Fig.1.

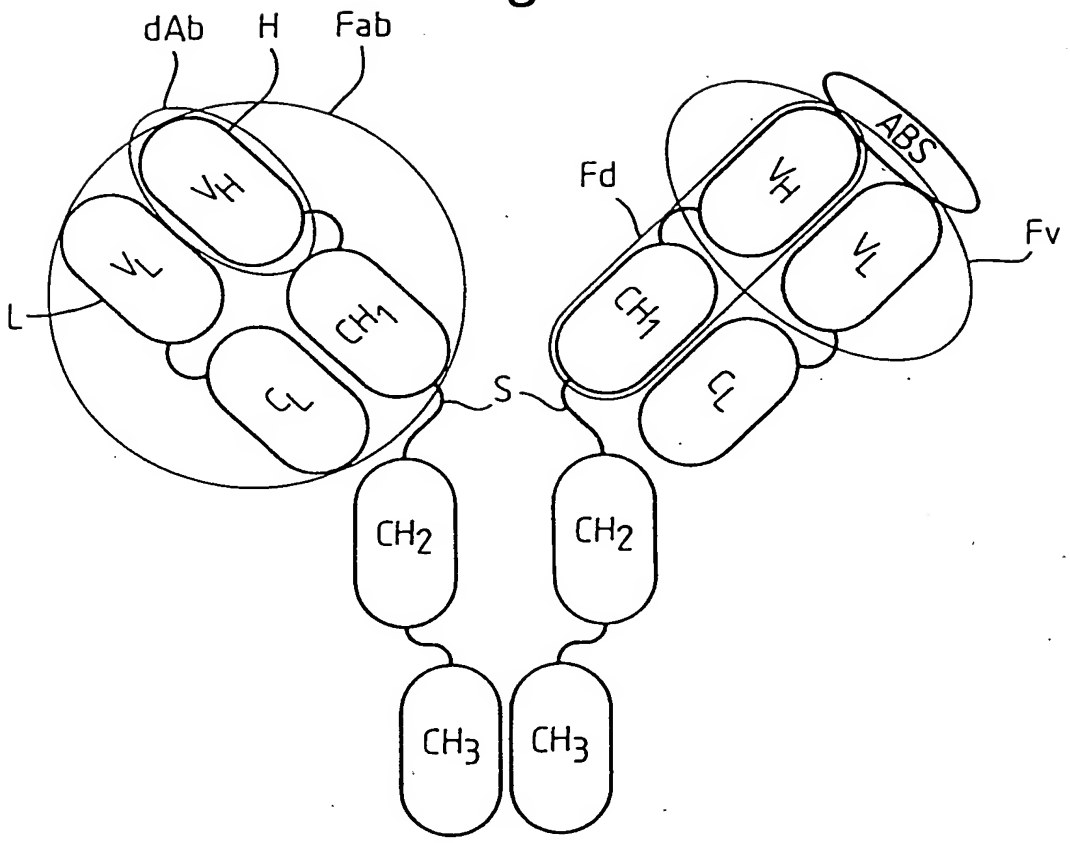


Fig.2a

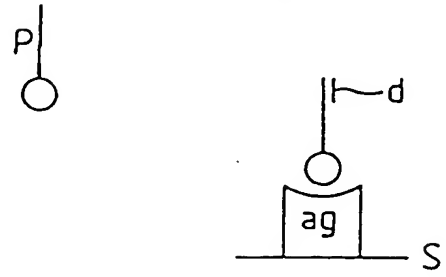


Fig.2b

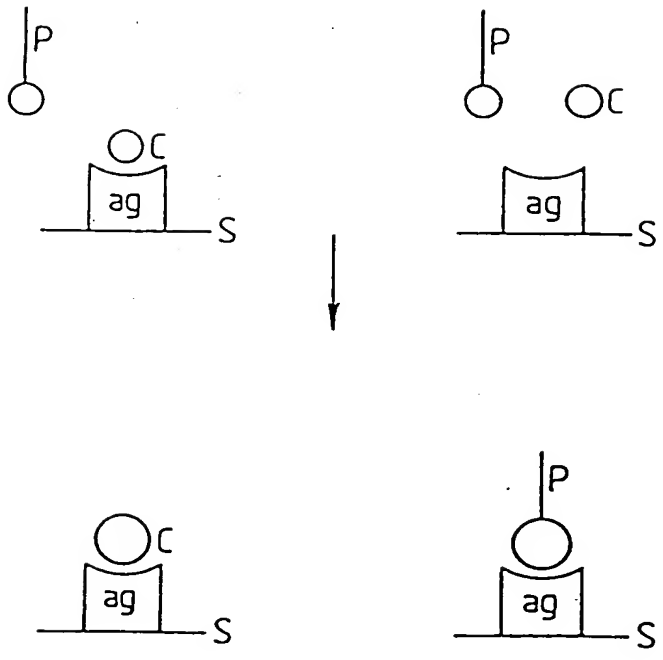
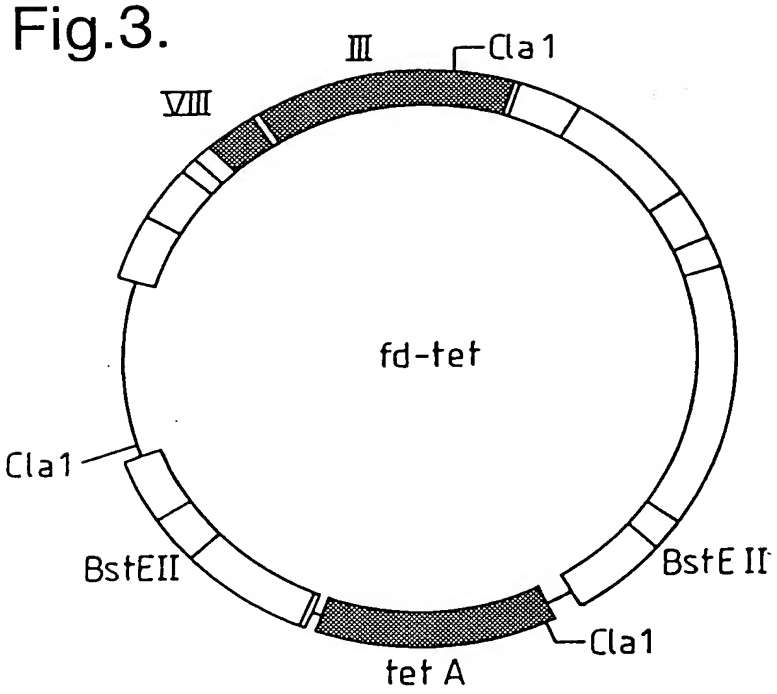


Fig.3.



fd - tet

⇓

cleave with BstEII

⇓

fill in with Klenow

⇓

re-ligate

↓

FDT δ Bst

⇓

in vitro mutagenesis (oligo 1)

↓

FDTPs/Bs

⇓

in vitro mutagenesis (oligo 2)

↓

FDTPs/Xh

Fig.4 a

(1653)
Oligo 1 ACA ACT TTC AAC AGT TGA GGA GAC GGT GAC CGT AAG CTT CTG CAG TTG GAC CTG AGC (SEQ ID NO. 177)
GGA GTG AGA ATA (1620)

(1653)
Oligo 2 ACA ACT TTC AAC AGT TTC CCG TTT GAT CTC GAG CTC CTG CAG TTG GAC CTG (SEQ ID NO. 178)

(1704)
Oligo 3 GTC GTC TTT CCA GAC GTT AGT (SEQ ID NO. 179)

Fig.4 b

GENE III

SIGNAL
CLEAVAGE SITE

GENE III

(1624)
A TCT CAC TCC GCT

(1650)
GAA ACT GTT GAA AGT (SEQ ID NO. 180)

Q V Q L Q (SEQ ID NO. 1) V T V S S (SEQ ID NO. 2)
B TCT CAC TCC GCT CAG GTC CAA CTG CAG AAG CTT ACG GTC ACC GTC TCC TCA ACT GTT GAA AGT (SEQ ID NO. 181)
PstI BstEII

Q V Q L Q (SEQ ID NO. 1) L E I K R (SEQ ID NO. 3)
C TCT CAC TCC GCT CAG GTC CAA CTG CAG GAG CTC GAG ATC AAA CGG GAA ACT GTT GAA AGT (SEQ ID NO. 182)
PstI XhoI

Fig.5a

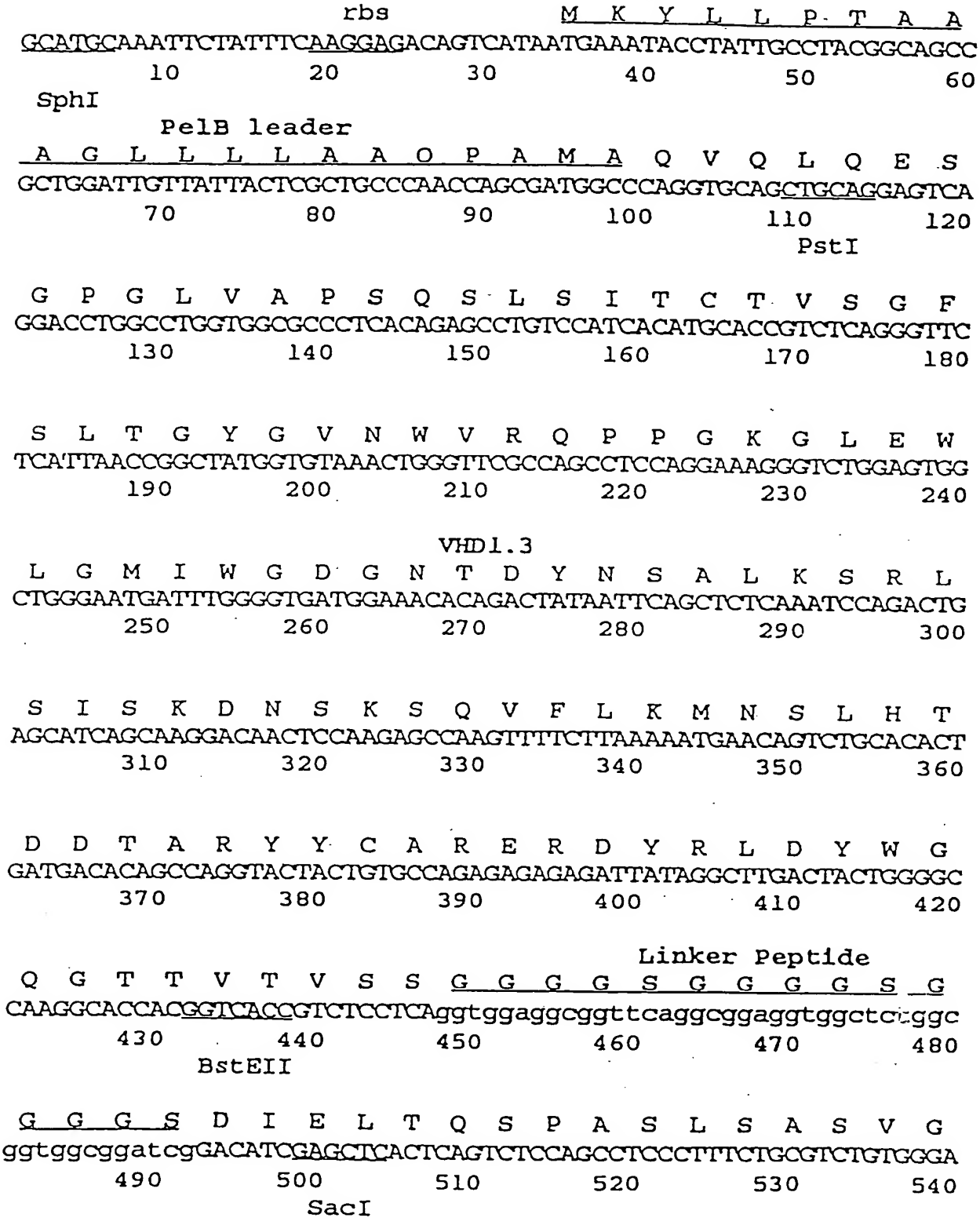

rbs
M K Y L L P T A A
GCATGCAAATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCC
10 20 30 40 50 60
SphI
PelB leader
A G L L L L A A Q P A M A Q V Q L Q E S
GCTGGATTGTTATTACTCGCTGCCCAACCAGCGATGGCCCAGGTGCAGCTGCAGGAGTCA
70 80 90 100 110 120
PstI
G P G L V A P S Q S L S I T C T V S G F
GGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACATGCACCGTCTCAGGGTTC
130 140 150 160 170 180
S L T G Y G V N W V R Q P P G K G L E W
TCATTAACCGGCTATGGTGTAAACTGGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGG
190 200 210 220 230 240
VHD1.3
L G M I W G D G N T D Y N S A L K S R L
CTGGGAATGATTTGGGGTGATGGAAACACAGACTATAATTCAGCTCTCAAATCCAGACTG
250 260 270 280 290 300
S I S K D N S K S Q V F L K M N S L H T
AGCATCAGCAAGGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCACACT
310 320 330 340 350 360
D D T A R Y Y C A R E R D Y R L D Y W G
GATGACACAGCCAGGTACTACTGTGCCAGAGAGAGAGATTATAGGCTTGACTACTGGGGC
370 380 390 400 410 420
Linker Peptide
Q G T T V T V S S G G G G S G G G G S G
CAAGGCACCACGGTCACCGTCTCCTCAggtggaggcggttcaggcggaggtggctctggc
430 440 450 460 470 480
BstEII
G G G S D I E L T Q S P A S L S A S V G
ggtggcggatcgGACATCGAGCTCACTCAGTCTCCAGCCTCCCTTTCTGCGTCTGTGGGA
490 500 510 520 530 540
SacI

Fig.5b

```
 E  T  V  T  I  T  C  R  A  S  G  N  I  H  N  Y  L  A  W  Y
GAAACTGTCACCATCACATGTCGAGCAAGTGGGAATATTCACAATTATTTAGCATGGTAT
       550        560        570        580        590        600

 Q  Q  K  Q  G  K  S  P  Q  L  L  V  Y  Y  T  T  T  L  A  D
CAGCAGAAACAGGGAAAATCTCCTCAGCTCCTGGTCTATTATACAACAACCTTAGCAGAT
       610        620        630        640        650        660

                                VKD1.3
 G  V  P  S  R  F  S  G  S  G  S  G  T  Q  Y  S  L  K  I  N
GGTGTGCCATCAAGGTTCAGTGGCAGTGGATCAGGAACACAATATTCTCTCAAGATCAAC
       670        680        690        700        710        720

 S  L  Q  P  E  D  F  G  S  Y  Y  C  Q  H  F  W  S  T  P  R
AGCCTGCAACCTGAAGATTTTGGGAGTTATTACTGTCAACATTTTTGGAGTACTCCTCGG
       730        740        750        760        770        780

                                                Myc Tag (TAG1)
 T  F  G  G  G  T  K  L  E  I  K  R  E  Q  K  L  I  S  E  E
ACGTTCGGTGGAGGGACCAAGCTCGAGATCAAACGGGAACAAAAACTCATCTCAGAAGAG
       790        800        810        820        830        840
                         XhoI

 D  L  N  *  *   (SEQ ID NO. 183)
GATCTGAATTAATAATGATCAAACGGTAATAAGGATCCAGCTCGAATTC  (SEQ ID NO. 184)
       850        860        870        880
                                              EcoRI
```

Fig.6.

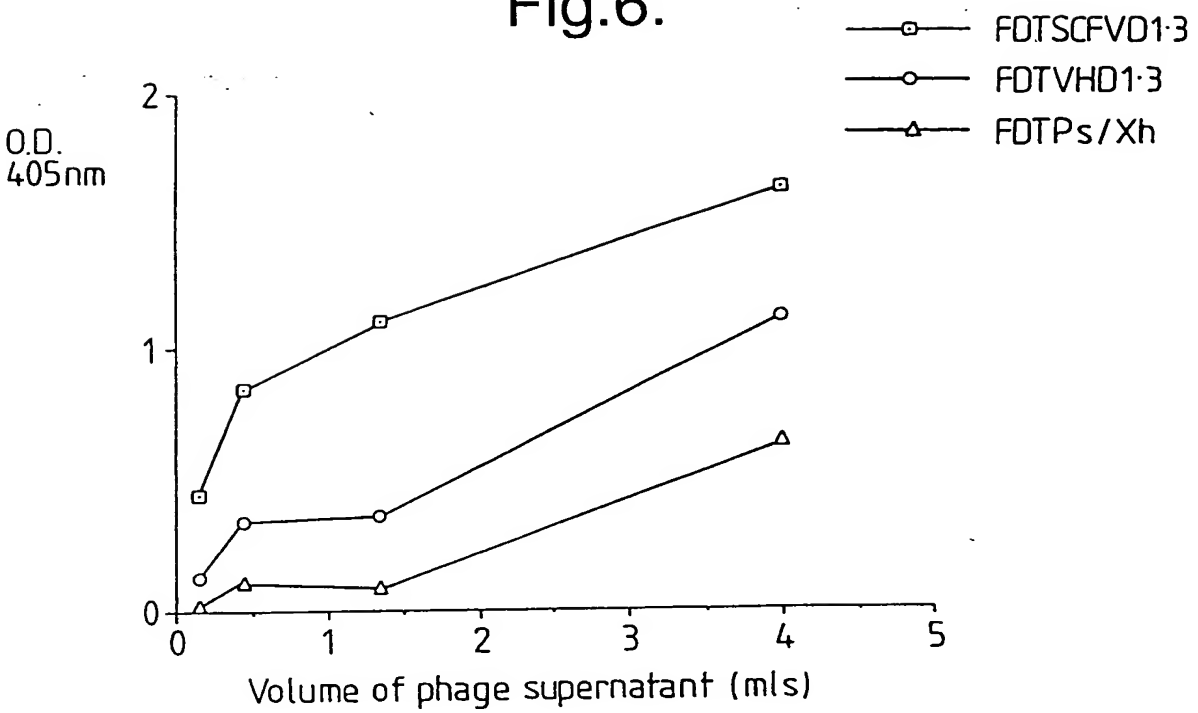

FDTSCFVD1·3
FDTVHD1·3
FDTPs/Xh
O.D.
405nm
2
1
0
0
1
2
3
4
5
Volume of phage supernatant (mls)

Fig.7.

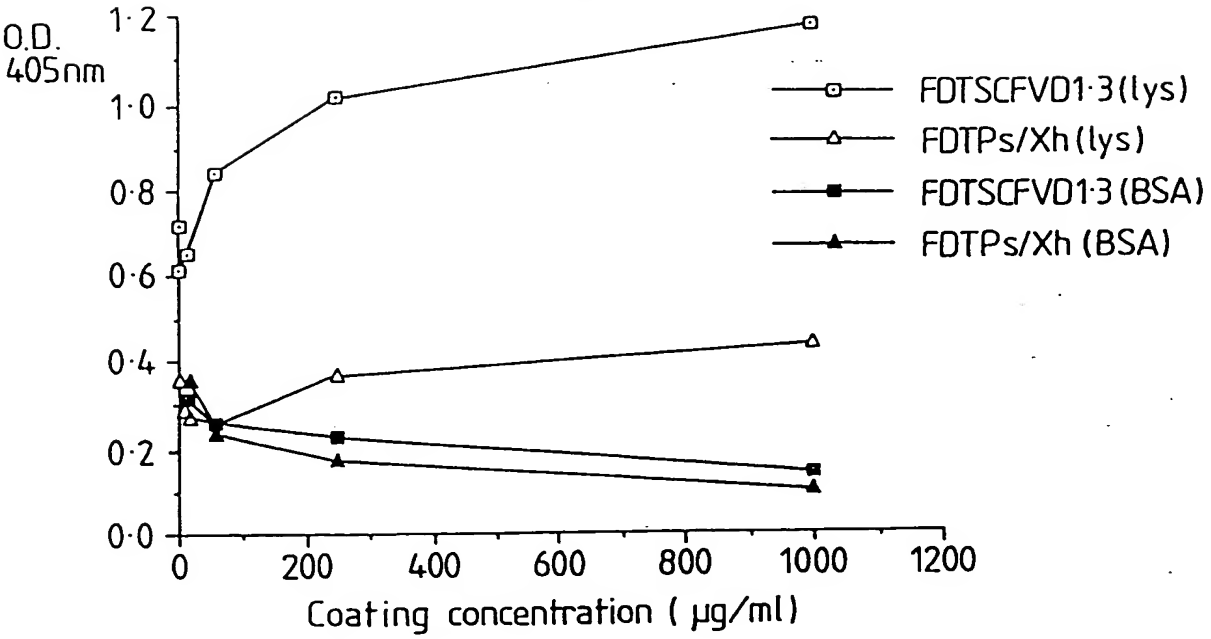

O.D.
405nm
1·2
1·0
0·8
0·6
0·4
0·2
0·0
FDTSCFVD1·3(lys)
FDTPs/Xh(lys)
FDTSCFVD1·3(BSA)
FDTPs/Xh (BSA)
0
200
400
600
800
1000
1200
Coating concentration (µg/ml)

Fig.8.

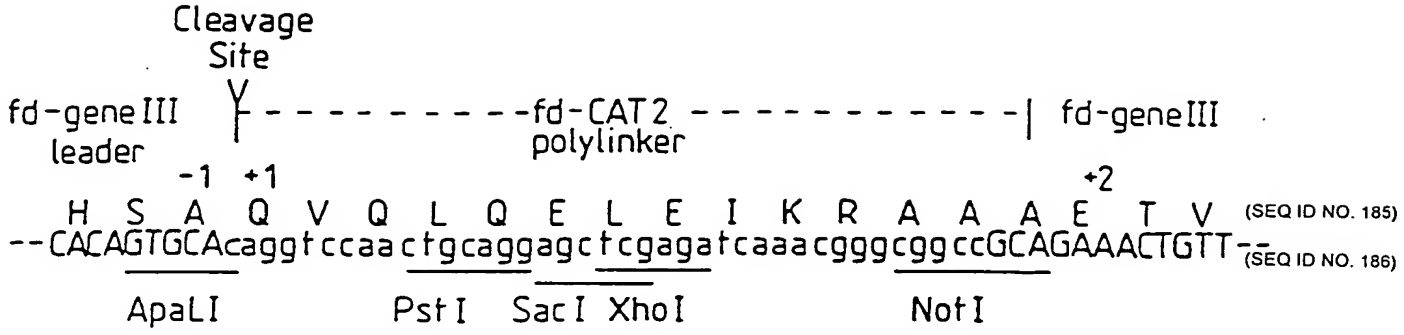

Cleavage Site
fd-gene III leader
fd-CAT2 polylinker
fd-gene III
-1 +1
+2
H S A Q V Q L Q E L E I K R A A A E T V (SEQ ID NO. 185)
--CACAGTGCAcaggtccaactgcaggagctcgagatcaaacgggcggccGCAGAAACTGTT-- (SEQ ID NO. 186)
ApaLI
PstI SacI XhoI
NotI

Fig.9.

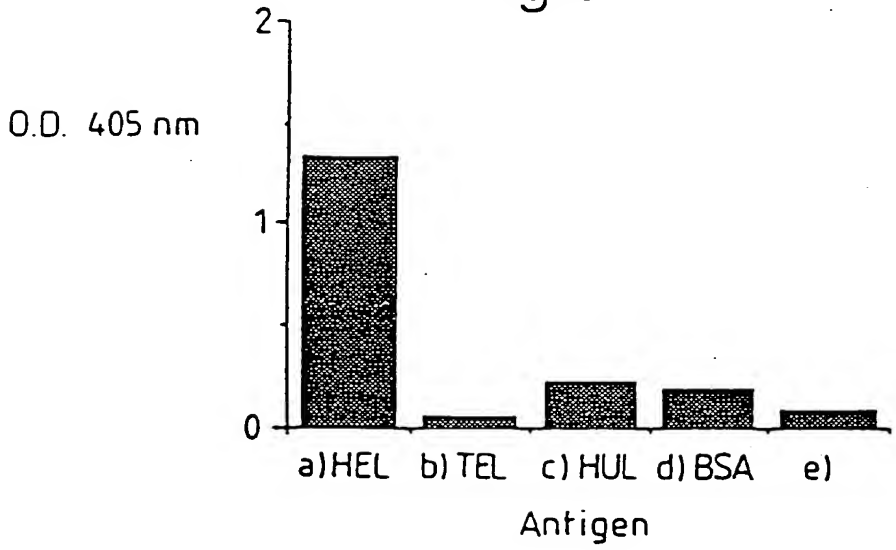

O.D. 405 nm
2
1
0
a) HEL
b) TEL
c) HUL
d) BSA
e)
Antigen

Fig.10a

```
                                                                M  K  Y  L  L  P  T  A  A
GCATGCAAATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCC
        10         20         30         40         50         60

 A  G  L  L  L  L  A  A  Q  P  A  M  A  Q  V  Q  L  Q  E  S
GCTGGATTGTTATTACTCGCTGCCCAACCAGCGATGGCCCAGGTGCAGCTGCAGGAGTCA
        70         80         90        100        110        120

 G  P  G  L  V  A  P  S  Q  S  L  S  I  T  C  T  V  S  G  F
GGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACATGCACCGTCTCAGGGTTC
       130        140        150        160        170        180

 S  L  T  G  Y  G  V  N  W  V  R  Q  P  P  G  K  G  L  E  W
TCATTAACCGGCTATGGTGTAAACTGGGTTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGG
       190        200        210        220        230        240

 L  G  M  I  W  G  D  G  N  T  D  Y  N  S  A  L  K  S  R  L
CTGGGAATGATTTGGGGTGATGGAAACACAGACTATAATTCAGCTCTCAAATCCAGACTG
       250        260        270        280        290        300

 S  I  S  K  D  N  S  K  S  Q  V  F  L  K  M  N  S  L  H  T
AGCATCAGCAAGGACAACTCCAAGAGCCAAGTTTTCTTAAAAATGAACAGTCTGCACACT
       310        320        330        340        350        360

 D  D  T  A  R  Y  Y  C  A  R  E  R  D  Y  R  L  D  Y  W  G
GATGACACAGCCAGGTACTACTGTGCCAGAGAGAGAGATTATAGGCTTGACTACTGGGGC
       370        380        390        400        410        420

 Q  G  T  T  V  T  V  S  S  A  S  T  K  G  P  S  V  F  P  L
CAAGGCACCACGGTCACCGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTG
       430        440        450        460        470        480

 A  P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D
GCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC
       490        500        510        520        530        540
```

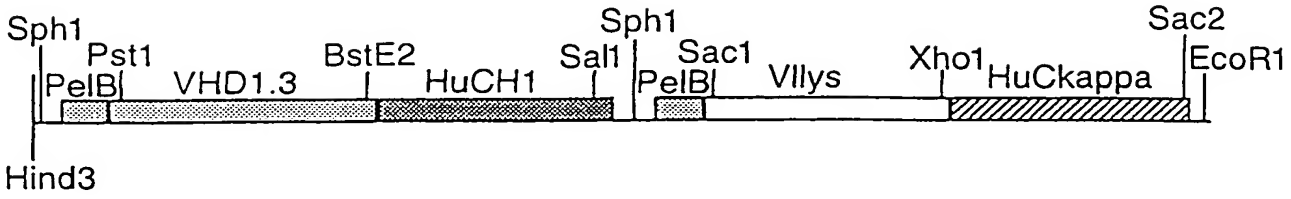
Fig.10b

```
 Y  F  P  E  P  V  T  V  S  W  N  S  G  A  L  T  S  G  V  H
TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC
      550        560        570        580        590        600

 T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V  V  T  V
ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG
      610        620        630        640        650        660

 P  S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K  P  S  N
CCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC
      670        680        690        700        710        720

 T  K  V  D  K  K  V  E  P  K  S  S  *  *   (SEQ ID NO. 187)
ACCAAGGTCGACAAGAAAGTTGAGCCCAAATCTTCATAATAACCCGGGAGCTTGCATGCA
      730        740        750        760        770        780

                                 M  K  Y  L  L  P  T  A  A  A  G  L
AATTCTATTTCAAGGAGACAGTCATAATGAAATACCTATTGCCTACGGCAGCCGCTGGAT
      790        800        810        820        830        840

    L  L  L  A  A  Q  P  A  M  A  D  I  E  L  T  Q  S  P  A  S
TGTTATTACTGCTGCCCAACCAGCGATGGCCGACATCGAGCTCACCCAGTCTCCAGCCT
      850        860        870        880        890        900

    L  S  A  S  V  G  E  T  V  T  I  T  C  R  A  S  G  N  I  H
CCCTTTCTGCGTCTGTGGGAGAAACTGTCACCATCACATGTCGAGCAAGTGGGAATATTC
      910        920        930        940        950        960

    N  Y  L  A  W  Y  Q  Q  K  Q  G  K  S  P  Q  L  L  V  Y  Y
ACAATTATTTAGCATGGTATCAGCAGAAACAGGGAAAATCTCCTCAGCTCCTGGTCTATT
      970        980        990       1000       1010       1020
```

Fig.10 c

```
   T  T  T  L  A  D  G  V  P  S  R  F  S  G  S  G  S  G  T  Q
ATACAACAACCTTAGCAGATGGTGTGCCATCAAGGTTCAGTGGCAGTGGATCAGGAACAC
      1030       1040       1050       1060       1070       1080

   Y  S  L  K  I  N  S  L  Q  P  E  D  F  G  S  Y  Y  C  Q  H
AATATTCTCTCAAGATCAACAGCCTGCAGCCTGAAGATTTTGGGAGTTATTACTGTCAAC
      1090       1100       1110       1120       1130       1140

   F  W  S  T  P  R  T  F  G  G  G  T  K  L  E  I  K  R  T  V
ATTTTTGGAGTACTCCTGGACGTTCGGTGGAGGCACCAAGCTGGAGATCAAACGGACTG
      1150       1160       1170       1180       1190       1200

   A  A  P  S  V  F  I  F  P  P  S  D  E  Q  L  K  S  G  T  A
TGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTG
      1210       1220       1230       1240       1250       1260

   S  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V  Q  W  K  V
CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGG
      1270       1280       1290       1300       1310       1320

   D  N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q  D  S  K  D
TGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGG
      1330       1340       1350       1360       1370       1380

   S  T  Y  S  L  S  S  T  L  T  L  S  K  A  D  Y  E  K  H  K
ACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACA
      1390       1400       1410       1420       1430       1440

   V  Y  A  C  E  V  T  H  Q  G  L  S  S  P  V  T  K  S  F  N
AAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA
      1450       1460       1470       1480       1490       1500

   R  G  E  S  *  *                    (SEQ ID NO. 188)
ACCGCGGAGAGTCATAGTAAGAATTC            (SEQ ID NO. 189)
      1510       1520
```

Fig.10 d



FabD1.3 in pUC19

Fig.11.

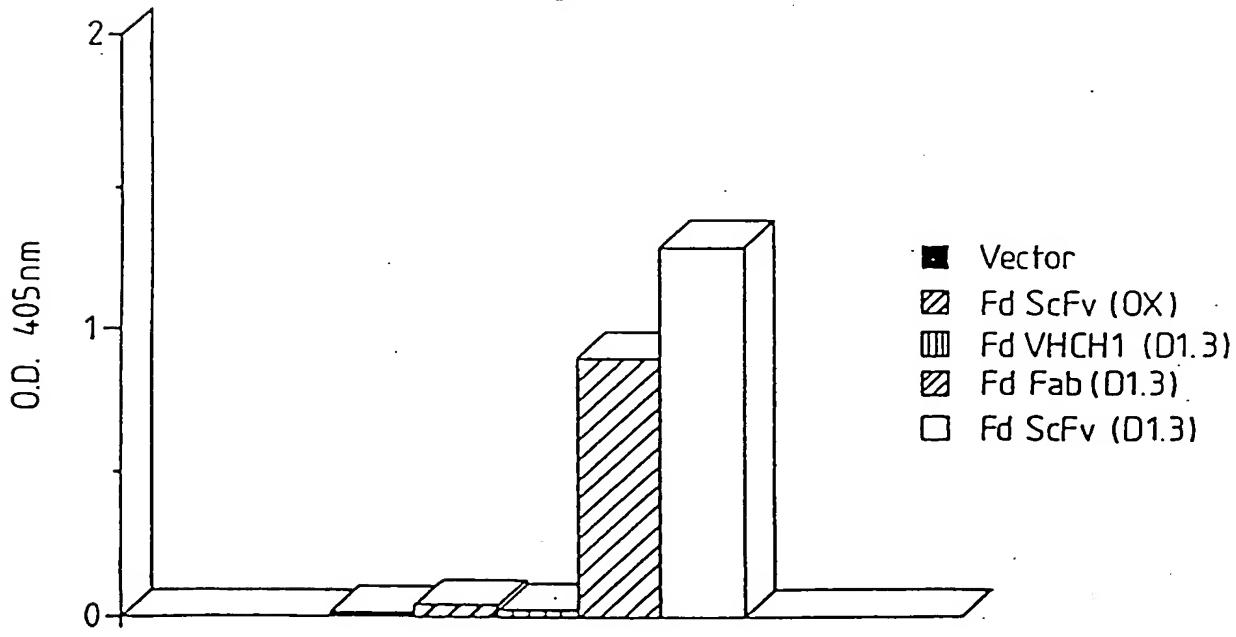


Fig.12a.

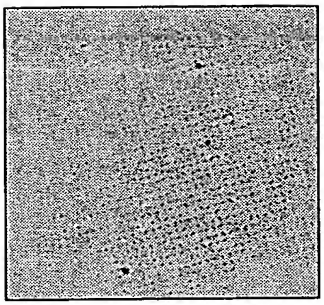


Fig.12b.

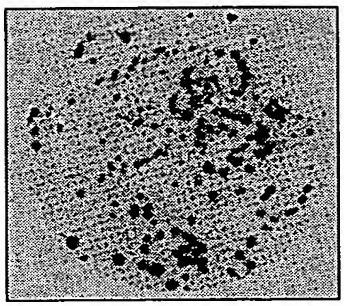


Fig.13.

```
 Q   V   Q   L   Q   E   S   G   G   G   L   V   Q   P   G   G
CAG GTG CAG CTG CAG GAG TCA GGA GGA GGC TTG GTA CAG CCT GGG GGT
                PstI
 S   L   R   L   S   C   A   T   S   G   F   T   F   S   N   Y
TCT CTG AGA CTC TCC TGT GCA ACT TCT GGG TTC ACC TTC AGT AAT TAC

 Y   M   G   W   V   R   Q   P   P   G   K   A   L   E   W   L
TAC ATG GGC TGG GTC CGC CAG CCT CCA GGA AAG GCA CTT GAG TGG TTG

 G   S   V   R   N   K   V   N   G   Y   T   T   E   Y   S   A
GGT TCT GTT AGA AAC AAA GTT AAT GGT TAC ACA ACA GAG TAC AGT GCA

 S   V   K   G   R   F   T   I   S   R   D   N   F   Q   S   I
TCT GTG AAG GGG CGG TTC ACC ATC TCC AGA GAT AAT TTC CAA AGC ATC

 L   Y   L   Q   I   N   T   L   R   T   E   D   S   A   T   Y
CTC TAT CTT CAA ATA AAC ACC CTG AGA ACT GAG GAC AGT GCC ACT TAT

 Y   C   A   R   G   Y   D   Y   G   A   W   F   A   Y   W   G
TAC TGT GCA AGA GGC TAT GAT TAC GGG GCC TGG TTT GCT TAC TGG GGC

 Q   G   T   L   V   T   v   s   s   g g g g s g g g g s
CAA GGG ACC CTG GTC ACC gtc tcc tca ggtggaggcggttcaggcggaggtggctct
             BstEII
 g g g g s d   i   E   L   T   Q   T   P   L   S   L   P   V
ggcggtggcggatcggac atc GAG CTC ACC CAA ACT CCA CTC TCC CTG CCT GTC
                      SacI
 S   L   G   D   Q   A   S   I   S   C   R   S   S   Q   S   I
AGT CTT GGA GAT CAA GCC TCC ATC TCT TGC AGA TCT AGT CAG AGC ATT

 V   H   S   N   G   N   T   Y   L   E   W   Y   L   Q   K   P
GTA CAT AGT AAT GGA AAC ACC TAT TTA GAA TGG TAC CTG CAG AAA CCA
                                            PstI
 G   Q   S   P   K   L   L   I   Y   K   V   S   N   R   F   S
GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA TTT TCT

 G   V   P   D   R   F   S   G   S   G   S   G   T   D   F   T
GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCG GGG ACA GAT TTC ACA

 L   K   I   S   R   V   E   A   E   D   L   G   V   Y   Y   C
CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TAC TGC

 F   Q   G   S   H   V   P   Y   T   F   G   G   G   T   K   L
TTT CAA GGT TCA CAT GTT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTC

 E   I   K   R
GAG ATC AAA CGG      (SEQ ID NO. 190)
XhoI                 (SEQ ID NO. 191)
```

Fig.14.

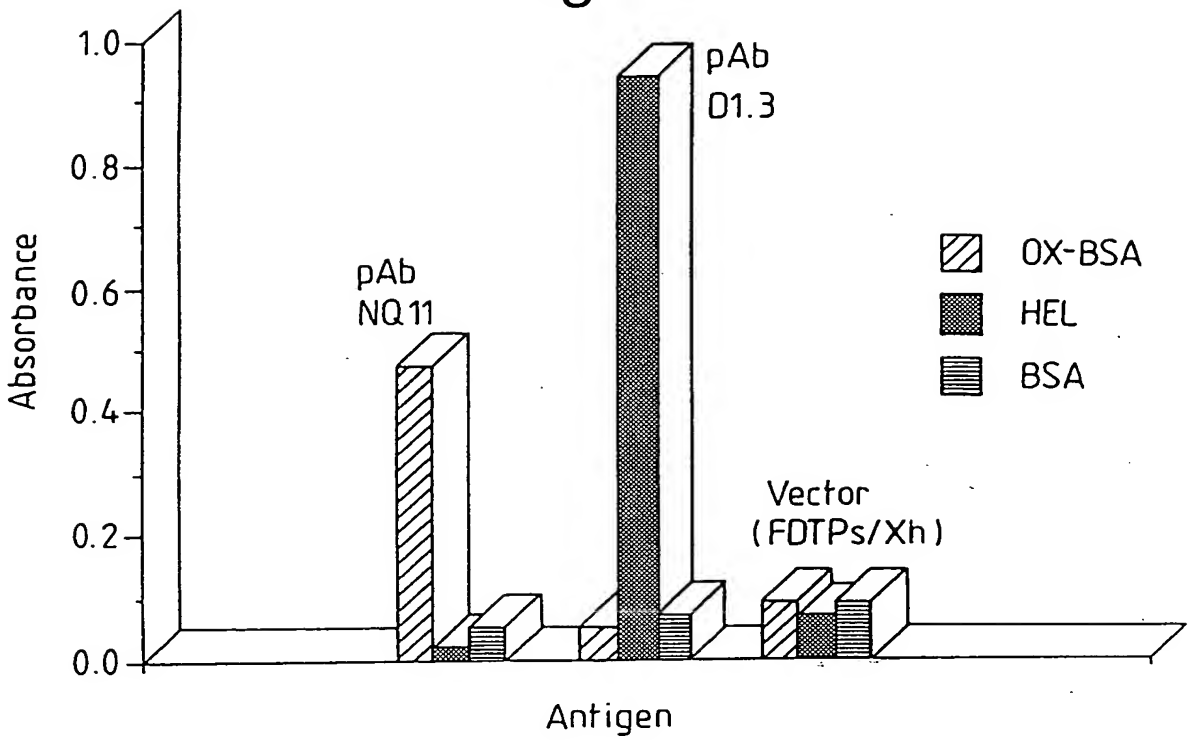


Fig.15.

5' END

R T P E M P V L (SEQ ID NO:192)

TCT CAC AGT GCA CAA ACT GTT GAA CGG ACA CCA GAA ATG CCT GTT CTG (SEQ ID NO:193)

ApaL1

3' END

K A A L G L K

AAA GCC GCT CTG GGG CTG AAA GCG GCC GCA GAA ACT GTT GAA AGT etc. (SEQ ID NO:194 (SEQ ID NO:195

Not I

Fig.16a

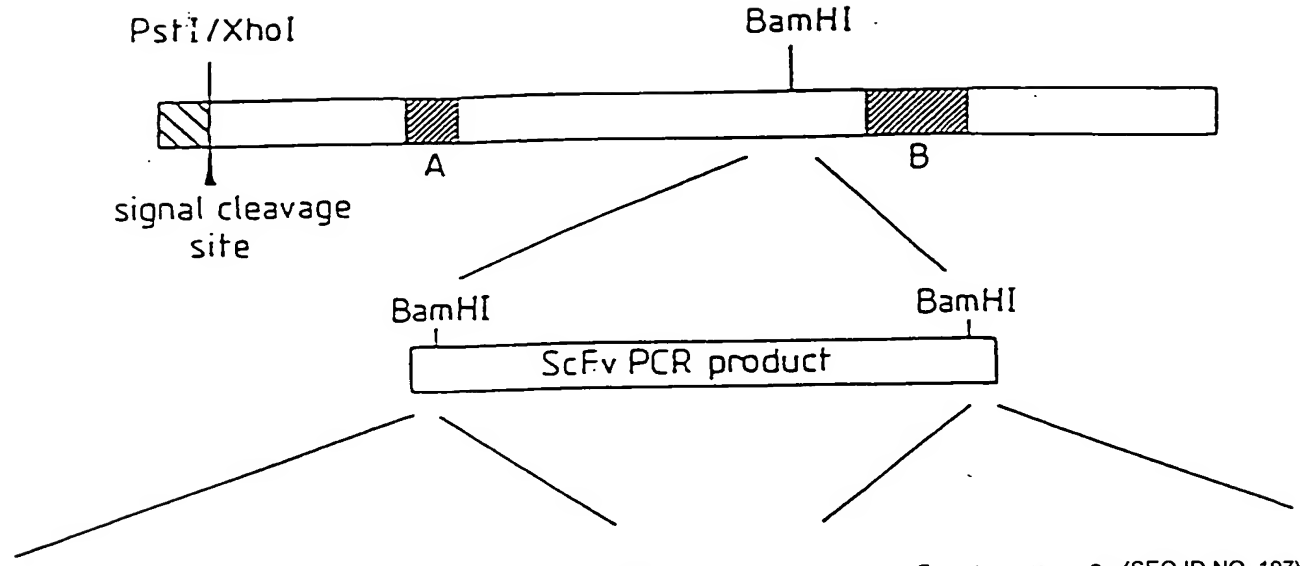


Fig.16b

A	(1834).5'	GAG GGT GGT GGC TCT		(SEQ ID NO. 200)
		- - -C - -		(SEQ ID NO. 201)
		- - -C - -		(SEQ ID NO. 202)
		- - -C - -	ACT 3'(1839)	(SEQ ID NO. 203)
B	(2284)	5' - GGC GGC GGC TCT		(SEQ ID NO. 204)
		- GGT GGT GGT -		(SEQ ID NO. 205)
		- - GGC GGC -		(SEQ ID NO. 206)
		GAG - - GGC -		(SEQ ID NO. 207)
		- - - GGT -		(SEQ ID NO. 208)
		- - - GGC -		(SEQ ID NO. 209)
		- - - GGT -		(SEQ ID NO. 210)
		- - - GGC -	3'(2379)	(SEQ ID NO. 211)

Reverse complement of mutagenic oligo G3Bamlink

5' GAG GGT GGC GGA TCC (SEQ ID NO. 212)

GAG GGT GGC GG 3' (SEQ ID NO. 213)

Fig.17.

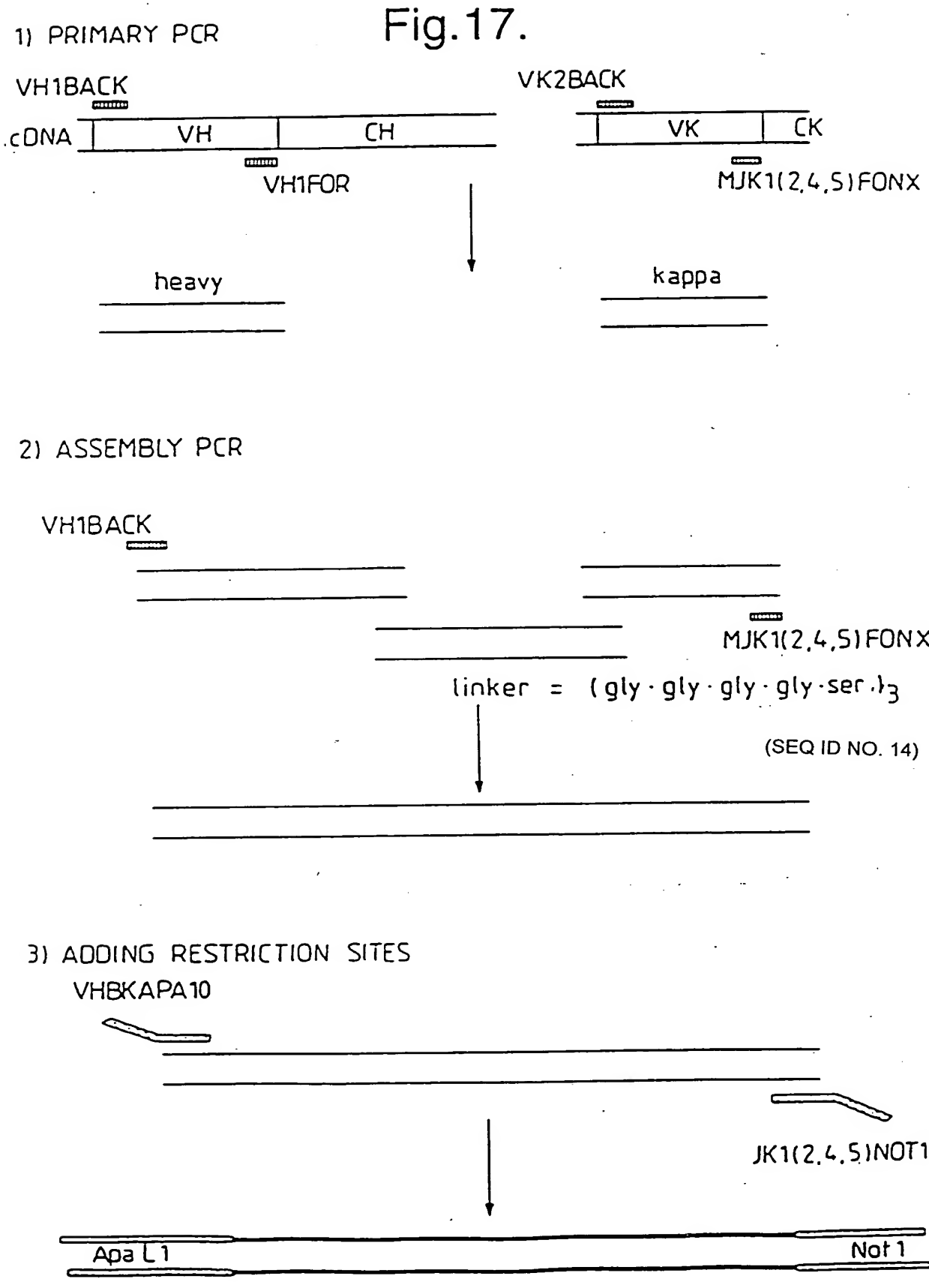

1) PRIMARY PCR
VH1BACK
VK2BACK
cDNA
VH
CH
VK
CK
VH1FOR
MJK1(2,4,5)FONX
heavy
kappa
2) ASSEMBLY PCR
VH1BACK
MJK1(2,4,5)FONX
linker = (gly·gly·gly·gly·ser·)3
(SEQ ID NO. 14)
3) ADDING RESTRICTION SITES
VHBKAPA10
JK1(2,4,5)NOT10
Apa L1
Not 1

Fig.18.

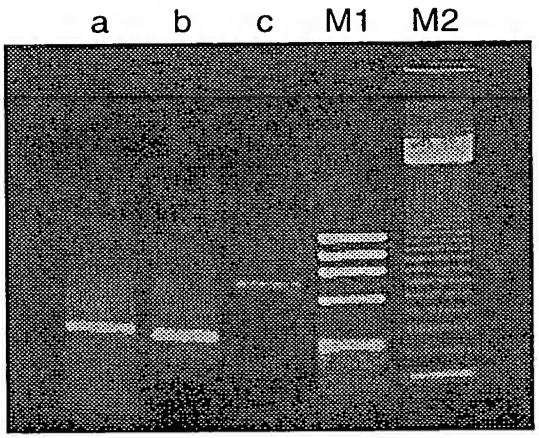


Fig.19.

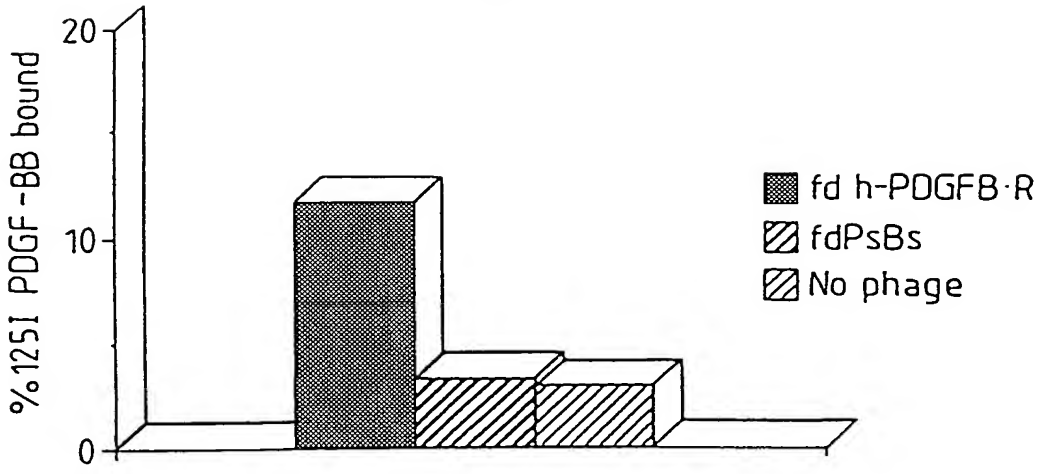

20
10
0
% 125I PDGF-BB bound
fd h-PDGFB·R
fdPsBs
No phage

Fig.20.

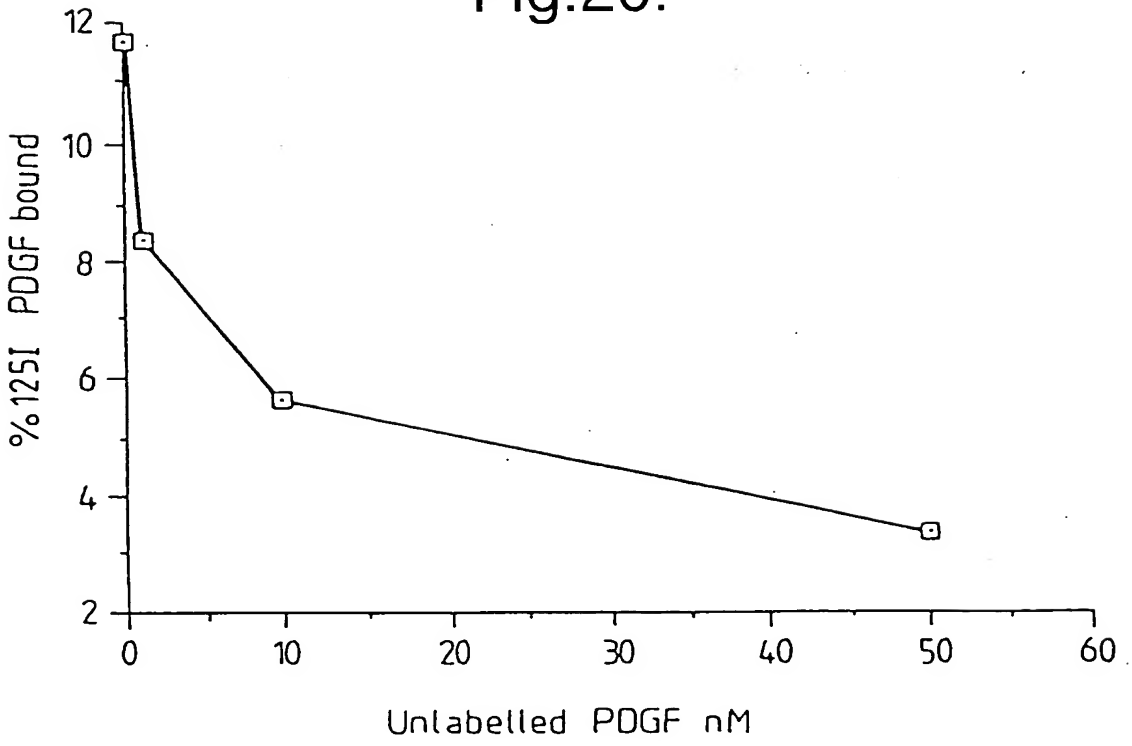

12
10
8
6
4
2
% 125I PDGF bound
0
10
20
30
40
50
60
Unlabelled PDGF nM

Fig.21.

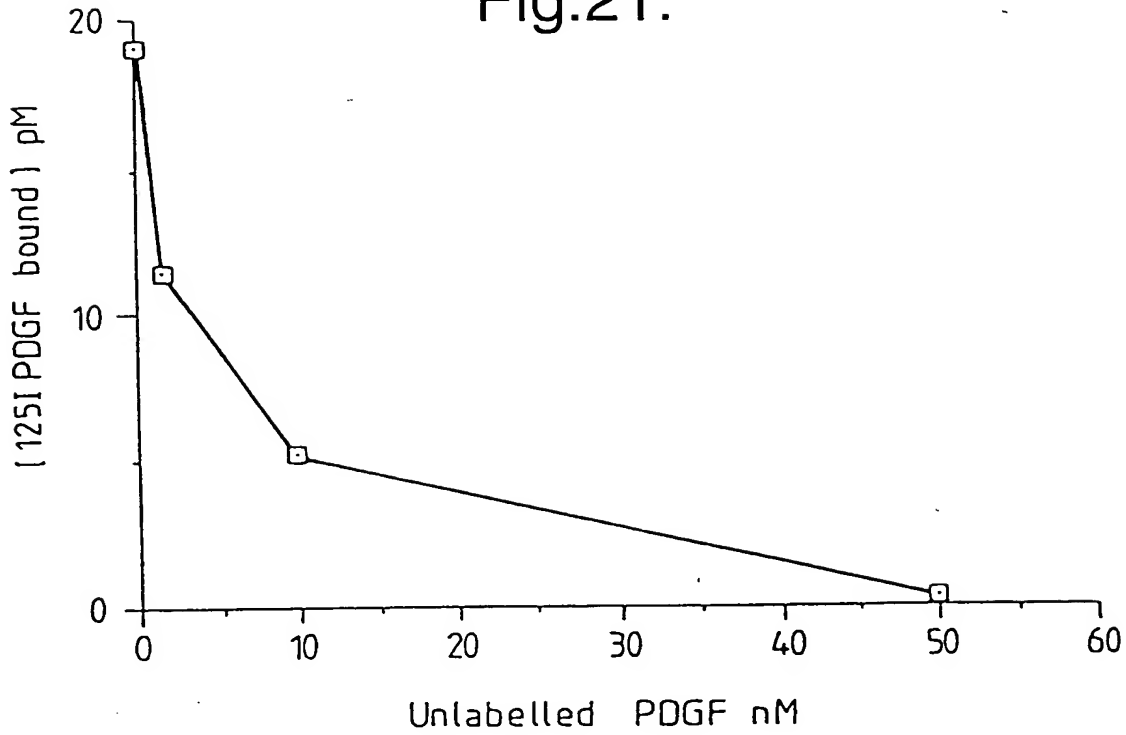

20
10
0
[125I PDGF bound] pM
0
10
20
30
40
50
60
Unlabelled PDGF nM

Fig.22.

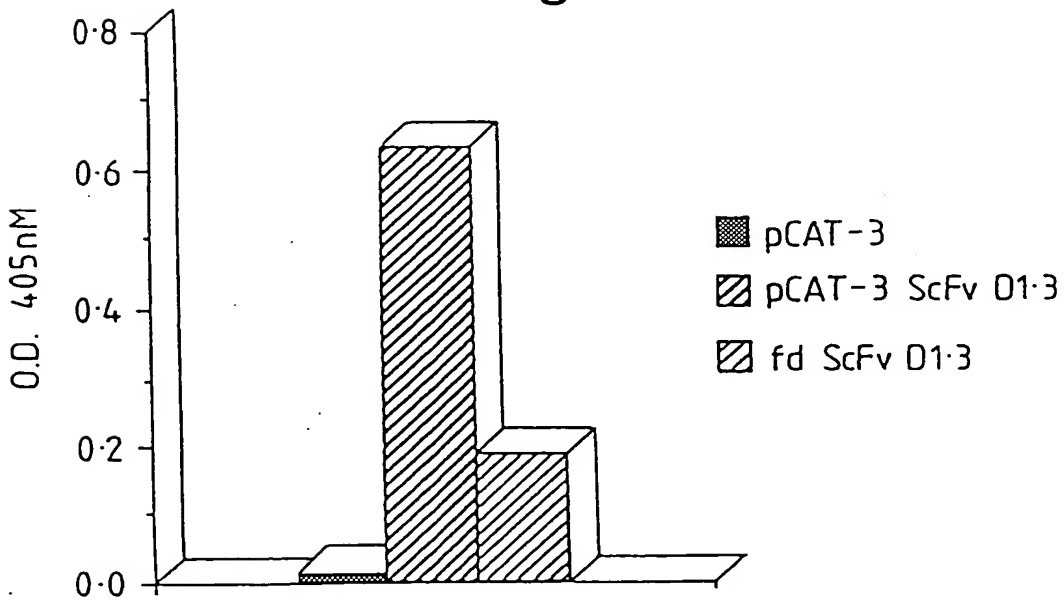

0·8
0·6
0·4
0·2
0·0
O.D. 405nM
pCAT-3
pCAT-3 ScFv D1·3
fd ScFv D1·3

Fig.23a

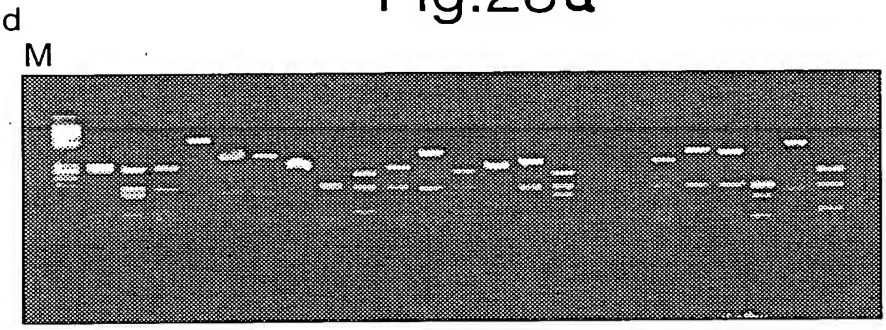
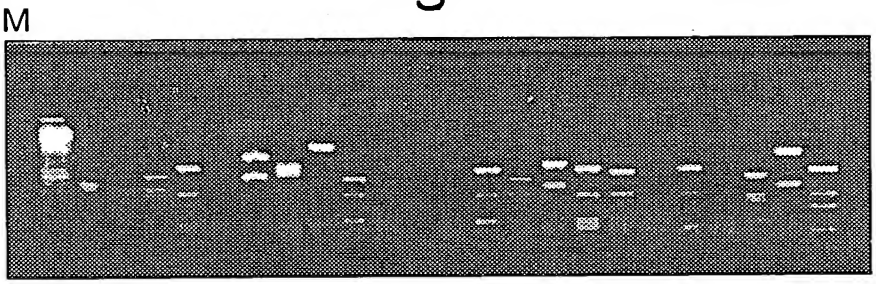


Fig.23b



VH sequences

Fig.24a

from combinatorial library:

		CDR1		CDR2		CDR3				
A	QVQLQQSGAELARPGASVKMSCKASGYTFT	SYTMH	WVKQRPGQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	RYGAY	WGQGTTVTVSS	x4	1	(SEQ ID NO. 214)
B	QVKLQQSGAELAKPGASVKMSCKASGYTFT	RDMHI	WLKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	NYGLY	WGQGTTVTVSS	x9	1	(SEQ ID NO. 215)
C	QVQLQQSGPELVKPGASVKMSCKASGYTFT	SYVMH	WVKQKPGQGLEWIG	YINPYNDGTKYNEKFKG	KATLTSDKSSSTAYMELSSLTSEDSAVYYCAI	YRSFPY	WGQGTTVTVSS	x3	1	(SEQ ID NO. 216)
D	QVQLQQSGPELVKPGASVKISCKASGYSFT	GYFMN	WVKQSHGKSLEWIG	RINPYNGDTFYNQKFKG	KATLTVDKSSSTAIMELLSLTSEDSAVYYCVG	ITTRFAY	WGQGTTVTVSS	x3	1	(SEQ ID NO. 217)
E	QVQLQESGPGLVAPSQSLSITCTVSGFSLT	SYGVH	WVRQPPGKGLEWLG	VIWAGGSTNYNSALMS	RLSISKDNSKSQVFLKMNSLQTDDTAMYYCAR	DRGDY	WGQGTTVTVSS		2	VH1ox1
F	QVQLQQSGPELAKPGASVKMSCKASGYTFT	SYLMH	WVKQRPGQGLKWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS		1	(SEQ ID NO. 219)
G	QVKLQQSGAELVRPGASVKLSCKASGYTFT	RYLMH	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	EATLTADKSSNTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS		1	(SEQ ID NO. 220)
H	QVQLQQSGPELMKPGASVKISCKASGYSFS	RYTMH	WVKQSHGKSLEWIG	YIAPFNGGTTYNQKFKG	KATLTVDRSSSTAYMQLSSLTSEDSAVYYCAT	DYGRD	WGQGTTVTVSS		1	(SEQ ID NO. 221)

from hierarchical library VH-rep x Vκ-d:

I	QVKLQQSGPELARPGVSVKMSCKASGYTFT	SYAMH	WVKQSQSKSLEWIG	VISTYNGNTNYNQKFKG	KATMTVDKSSSTAYMELARLTSEDSAIYYCAR	DYGDY	WGQGTTVTVSS		1	(SEQ ID NO. 222)
J	QVKLQQSGAELARPGASVKMSCKASGYTFT	RYTMH	WVKQRPGQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DRGAY	WGQGTTVTVSS		1	(SEQ ID NO. 223)
K	QVKLQQSGAELAKPGASVKMSCKASGYTFT	RDMHI	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	NYGLY	WGQGTTVTVSS	x3	1	(SEQ ID NO. 224)
L	QVQLQQSGLELAKPGASVKMSCKASGYTFT	NYLMH	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS	x2	1	(SEQ ID NO. 225)
M	QVKLQQSGAELAKPGASVKMSCKASGYTFT	NYMMH	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSDDSAVYYCAR	DYGYF	WGQGTTVTVSS		1	(SEQ ID NO. 226)
N	QVQLQQSGAELVKPGASVKLSCKTSGYTFT	SYTMH	WVKQRPGQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS		1	(SEQ ID NO. 227)
O	QVQLQQSGAELAKPGASVKMSCEASGYTFT	SHLMH	WVKQRPGQGLEWIG	YINPATGYTEYNQKFKD	KATFTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGAY	WGQGTTVTVSS		1	(SEQ ID NO. 228)
P	QVKLQQSGAELAKPGASVKMSCKASGYTFT	SYWMH	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS		1	(SEQ ID NO. 229)
Q	QVKLQQSGAELAKPGASVKMSCKATGYTFT	SYLMH	WVKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS		1	(SEQ ID NO. 230)
R	QVQLQQSGAELAKPGASVKMSCKASGYTFT	SYVMH	WVKQRPGQGLEWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	NYGIY	WGQGTTVTVSS		1	(SEQ ID NO. 231)
S	QVQLQQSGAELAKPGASVKMSCKASGYTFT	TFLMH	WLKQRPGQGLEWIG	YINPSTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS	x2	1	(SEQ ID NO. 232)
T	QVKLQQSGAELARPGASVKMSCKASGYTFT	SYTMH	WVKQRPGQGLGWIG	YINPSSGYTNYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS	x6	1	(SEQ ID NO. 233)
U	QVKLQQSGAELAKPGASVKMSCKASGYTFT	SYTMH	WVKQRPGQGLEWIG	YIIPTTGYTEYNQKFKD	KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR	DYGYY	WGQGTTVTVSS		1	(SEQ ID NO. 234)
B	*QVKLQQSGAELAKPGASVKMSCKASGYTFT*	*RDMHI*	*WLKQRPGQGLEWIG*	*YINPSTGYTEYNQKFKD*	*KATLTADKSSSTAYMQLSSLTSEDSAVYYCAR*	*NYGLY*	*WGQGTTVTVSS*			(SEQ ID NO. 235)

Fig.24b

Vκ sequences

from combinatorial library:

		CDR1		CDR2		CDR3					
a	DIELTQSPSSLSASLGERVSLTC	RASQEISGYLS	WLQQKPDGSIKRLIY	AASTLES	GVPKRFSGSRSGSDYSLTISSLESEDFADYYC	LQYASYPT	FGAGTKLEIKRA	x3	V	ox-like	(SEQ ID NO. 236)
b	DIELTQSPAIMSASPGEKVTMTC	RASSSVSSSYLH	WYQQKSGASPKVWIY	STSNLAS	GVPARFSGSGSGTSYSLTISSVEAEDAATYYC	QQYSGYPLT	FGAGTKLEIKRA	x3	IV	ox-like	(SEQ ID NO. 237)
c	DIELTQSPTTMAASPGEKITITC	SASSSISSNYLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSSIPLT	FGAGTKLEIKRA	x3	IV	ox-like	(SEQ ID NO. 238)
d	DIELTQSPTTMAASPGEKITITC	SASSSISSNYLH	WFQQKPGFSPKLLIS	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMSAEDVATYYC	QQGSTIPFT	FGSGTKLEIKRA	x9	IV	ox-like	(SEQ ID NO. 239)
e	DIELTQSPAIMSASPGEKVTITC	SASSSVNYMH	WFQQKPGTSPKLWIY	STSNLAS	GVPTRFSGSGSGTSYSLTISRMEAEDAATYYC	QQRSSYPPT	FGSGTKLEIKRA	x4	VI	ox-like?	(SEQ ID NO. 240)
f	DIELTQSPAIMSAFPGEKVTMTC	SASSSVSYMN	WYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQFSSNPLT	FGAGTKLELKRA		VI	Vκox1	(SEQ ID NO. 241)
g	DIELTQSPAIMSASPGEKVTMTC	SASSSINYMH	WYQQKPGASPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	HQRNSYPWT	FGGGTKLEIKRA		VI	ox-like?	(SEQ ID NO. 242)

from hierarchical library VH-B x Vκ-rep:

h	DIELTQSPAIMSASPGEKVTMTC	SASSSVSYMH	WYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQWGSNPLT	FGAGTKLEIKRA	x4	IV/VI	Vκox1	(SEQ ID NO. 243)
i	DIELTQSPAIMSASPGEKVTITC	SASSSVSYIH	WFQQKPGTSPKLWIY	STSNLAS	GVPARFSGSGSGTSYSLTISRMEAEDAATYYC	QQYHSYPLT	FGAGTKLEIKRA		V	ox-like?	(SEQ ID NO. 244)
j	DIELTQSPTTMAASPGEKITITC	SASSSISSNYLH	WFQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSSIPLT	FGGGTKLEIKRA		V	ox-like	(SEQ ID NO. 245)
k	DIELTQSPTTMAASPGDMITITC	SATSGISSNYLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPPRFSGSGSGTGYSLTIGAMEAEDVATYYC	QQGSSIPYT	FGAGTKLEIKRA		V	ox-like	(SEQ ID NO. 246)
l	DIELTQSPTTMAASPGEKITITC	SASSSISSNYLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSSIPYT	FGGGTKLEIKRA		V	ox-like	(SEQ ID NO. 247)
m	DIELTQSPTTMAASPGEKITITC	SASSSISSNHLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTGYSLTIGTMEAEDVATYYC	QQGSGIPYT	FGGGTKLEIKRA		V	ox-like	(SEQ ID NO. 248)
n	DIELTQSPTTMAASPGEKITITC	SASSSISSNYLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSSIPFT	FGGGTKLEIKRA		V	ox-like	(SEQ ID NO. 249)
o	DIELTQSPAIMAASPGEKITITC	SASSSISSNYLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSSIPYT	FGGGTKLEIKRA	x2	V	ox-like	(SEQ ID NO. 250)
p	DIELTQSPAIMSASPGEKVTMTC	SASSSVSYMH	WYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDVATYYC	QQWSSNPLT	FGAGTKLEIKRA	x2	IV/VI	Vκox1	(SEQ ID NO. 251)
q	DIELTQSPAIMSASPGDKVTLTC	SASSSVRYVN	WFQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTGYSLTISSMEAEDAATYYC	QQWTSNPPT	FGGGTKLEIKRA		IV/VI	Vκox1	(SEQ ID NO. 252)
r	DIELTQSPAIMSASPGEKVTMTC	SASSSVSYMH	WYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQWSTNALT	FGAGTKLEIKRA		IV/VI	Vκox1	(SEQ ID NO. 253)
s	DIELTQSPAIMSASPGEKVTMTC	RASSSVTSSYLN	WYQQKSGASPKLWVY	STSNLAS	GVPARFSGSGSGTSYSLTISSVEAEDAATYYC	QQYSGYPLT	FGAGTKLEIKRA		IV/VI	ox-like	(SEQ ID NO. 254)
t	DIELTQSPAIMSASPGEKVTMTC	RASSSVSSSYLN	WYQQKSGASPKLWIY	STSNLAS	GVPARFSGSGSGTSYSLTISRMEAEDAATYYC	QQRSSYPLT	FGAGTKLEIKRA		IV/VI	ox-like	(SEQ ID NO. 255)
u	DIELTQSPAIMSASPGEKVTMTC	RASSSVSSSYLH	WYQQKSGASPKLWIY	STSNLAS	GVPARFSGSGSGTSYSLTISSVEAEDAATYYC	QQYSGYPLT	FGAGTKLEIKRA		IV/VI	ox-like	(SEQ ID NO. 256)
v	DIELTQSPAIMSASPGEKVTMTC	RASSSVSSSYLH	WFQQKSGASPKLWIY	STSNLPS	GVPARFSGSGSGTSYSLTISSVEAEDAATYYC	QQYSGYPLT	FGGGTKLEIKRA		IV/VI	ox-like	(SEQ ID NO. 257)
c	DIELTQSPTTMAASPGEKITITC	SASSSISSNYLH	WYQQKPGFSPKLLIY	RTSNLAS	GVPARFSGSGSGTSYSLTIGTMEAEDVATYYC	QQGSSIPLT	FGAGTKLEIKRA	x3			(SEQ ID NO. 258)

Fig.25.

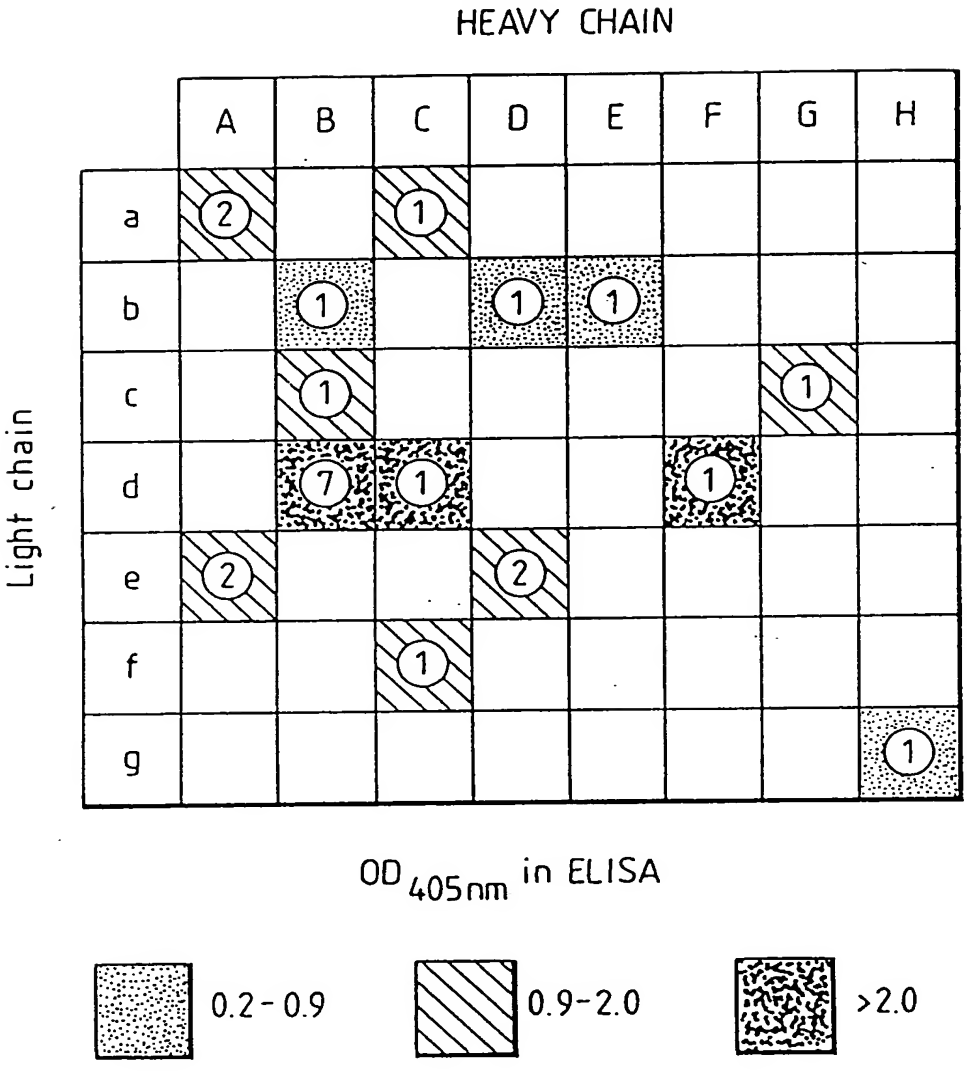

HEAVY CHAIN
A
B
C
D
E
F
G
H
a
b
c
d
e
f
g
Light chain
2
1
1
1
1
1
7
1
1
2
2
1
1
OD 405nm in ELISA
0.2 - 0.9
0.9-2.0
>2.0

Fig.26(a).

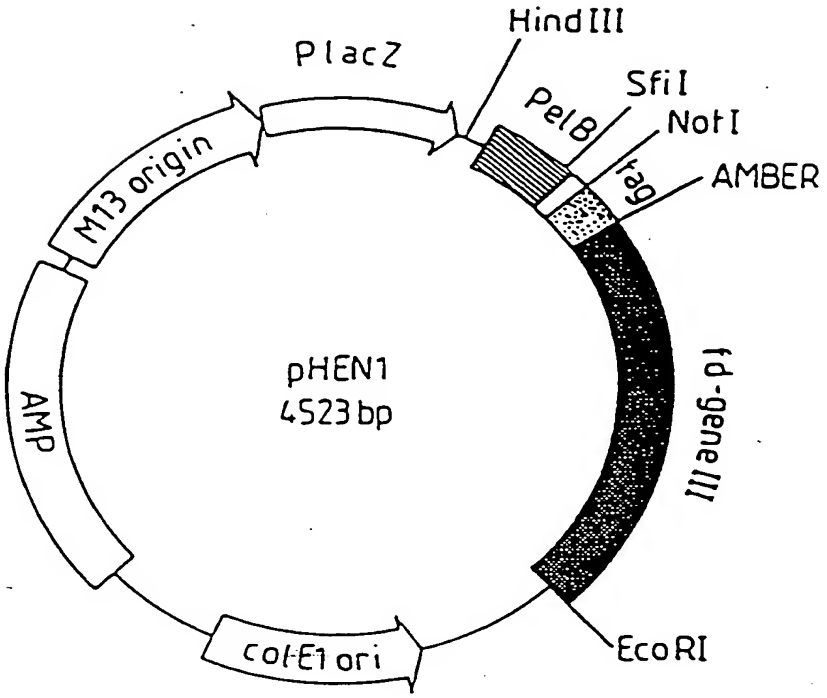


Fig.26(b).

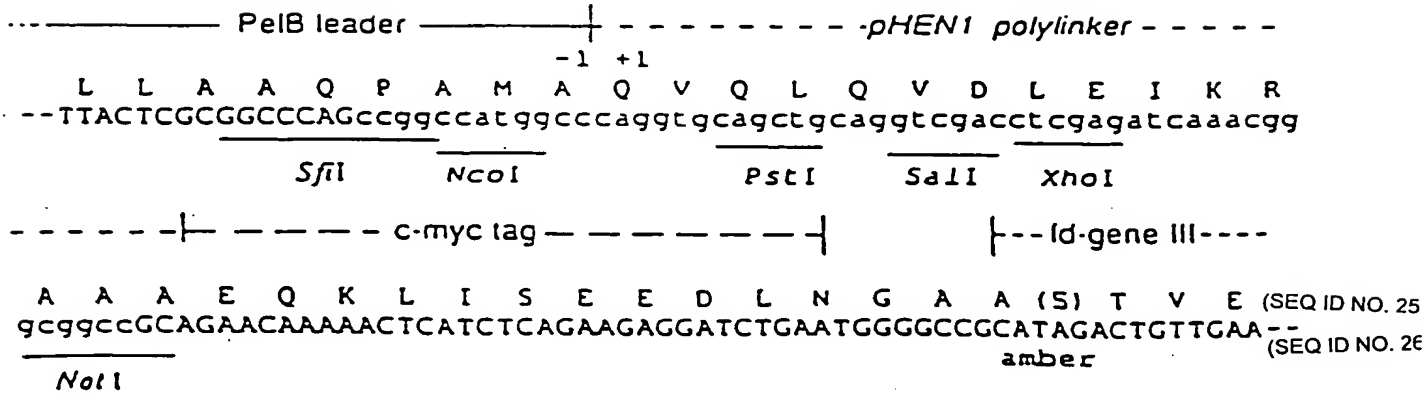


Fig.27.

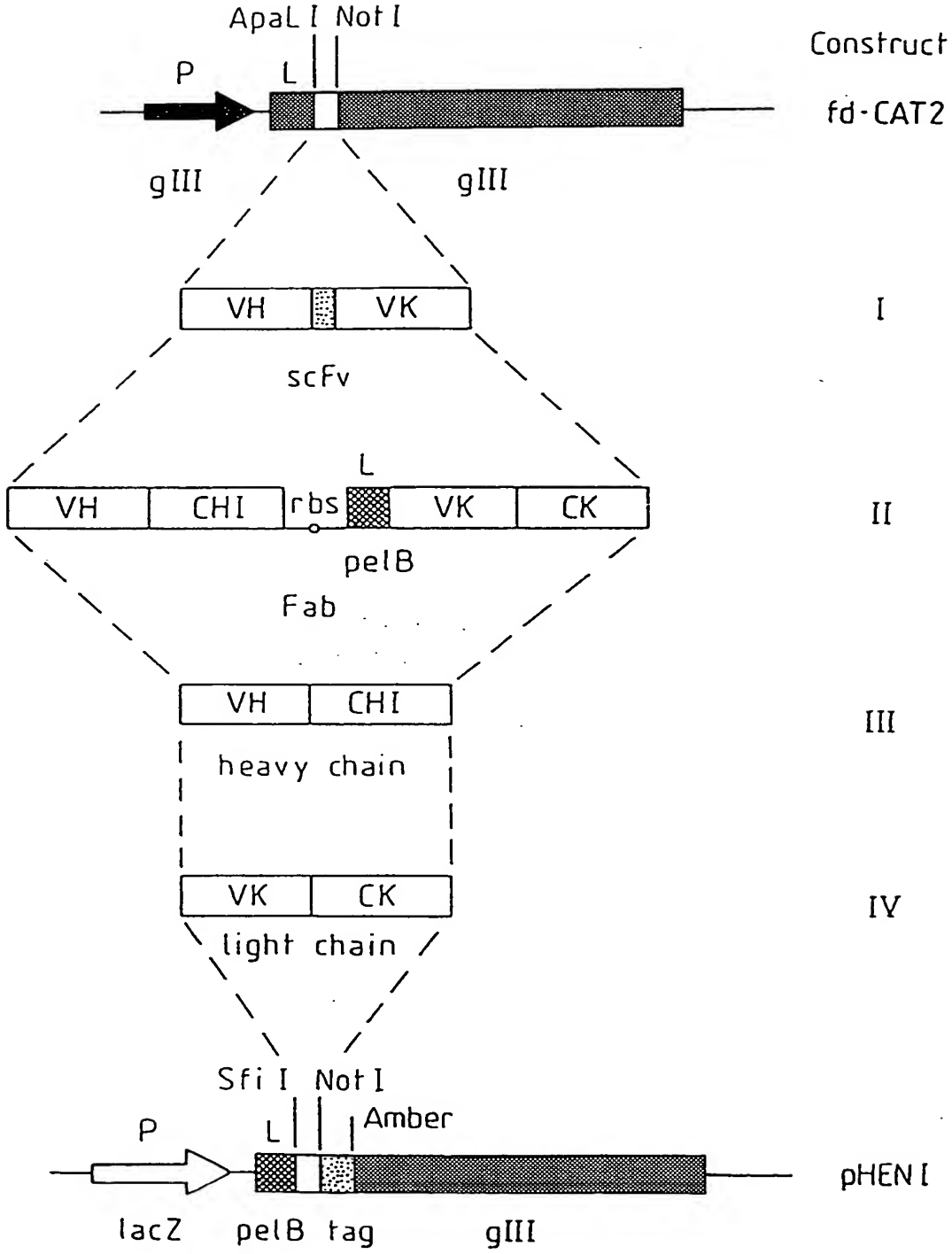

ApaL I
Not I
Construct
P
L
fd-CAT2
gIII
gIII
VH
VK
I
scFv
L
VH
CHI
rbs
VK
CK
II
pelB
Fab
VH
CHI
III
heavy chain
VK
CK
IV
light chain
Sfi I
Not I
Amber
P
L
pHEN I
lacZ
pelB
tag
gIII

Fig.28.

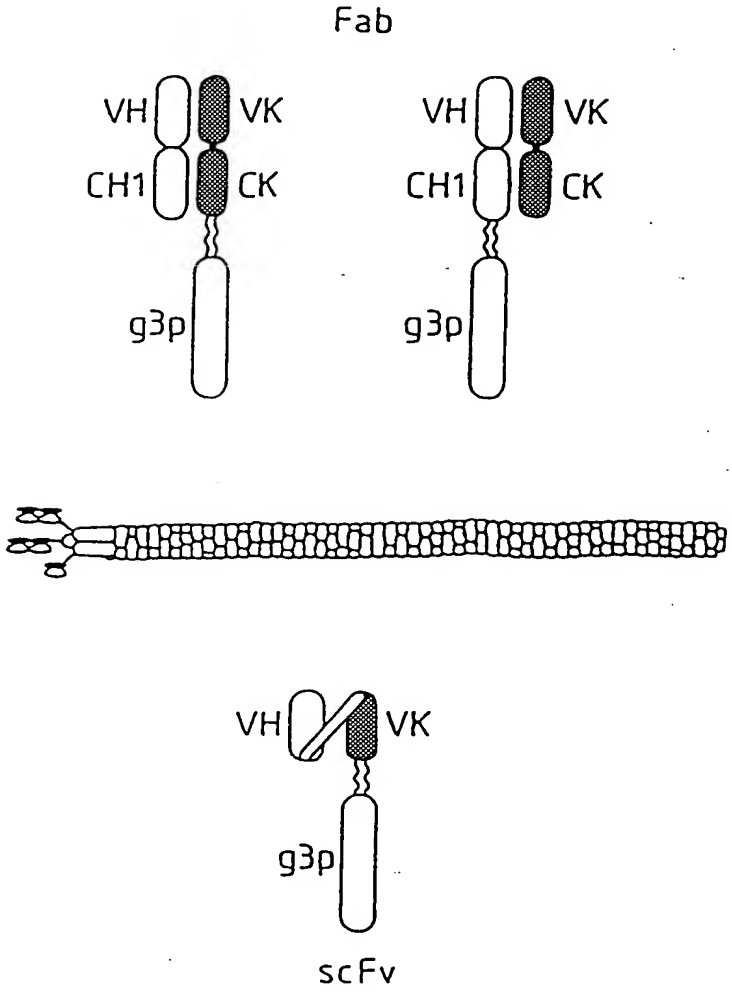


Fig.29.

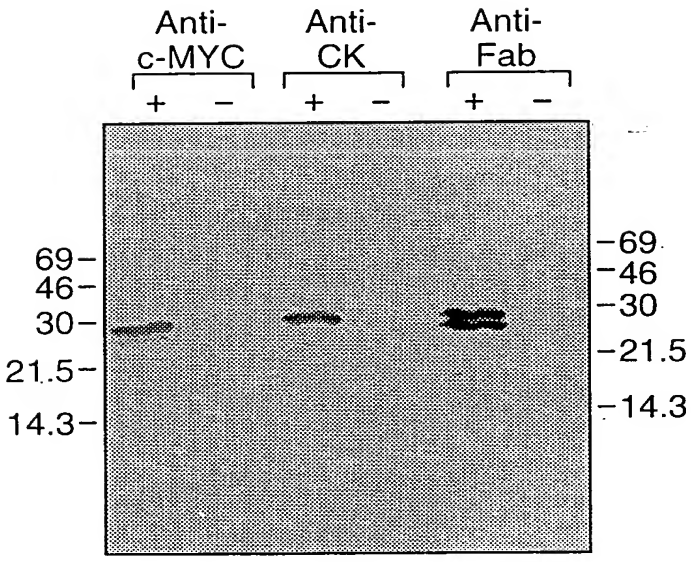


Fig.30.

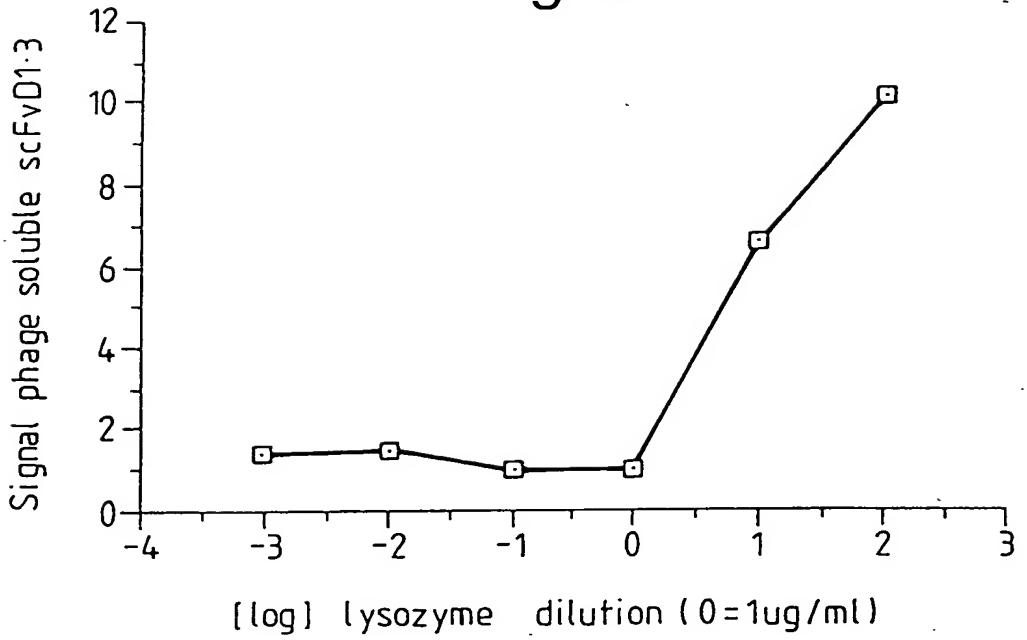

Signal phage soluble scFvD1·3
12
10
8
6
4
2
0
-4
-3
-2
-1
0
1
2
3
[log] lysozyme dilution (0=1ug/ml)

Fig.31.

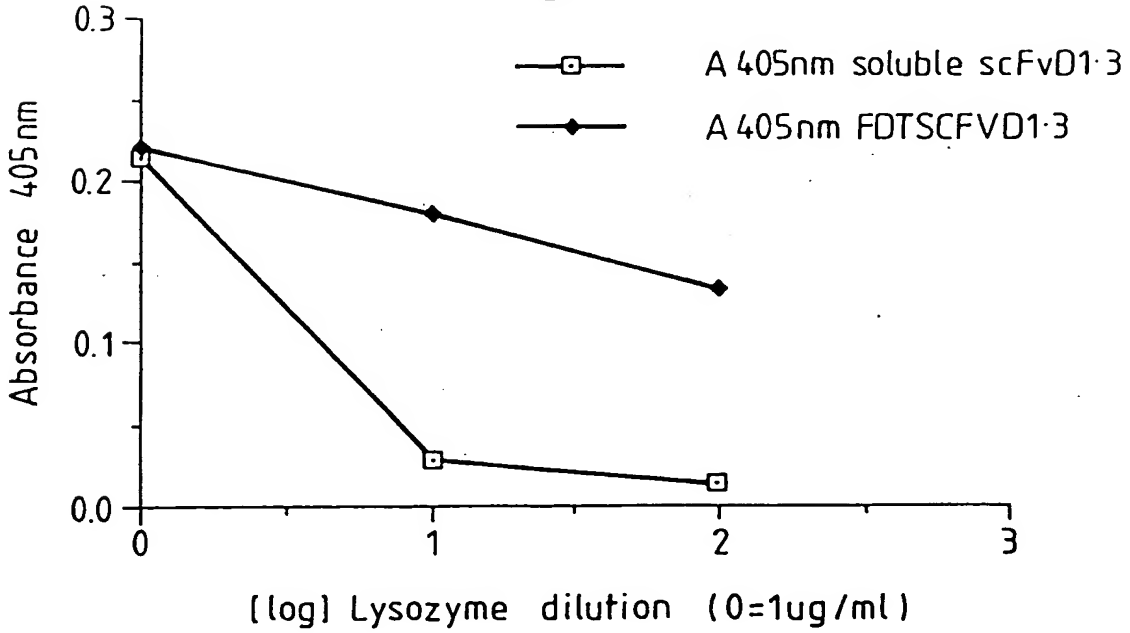

A 405nm soluble scFvD1·3
A 405nm FDTSCFVD1·3
Absorbance 405nm
0.3
0.2
0.1
0.0
0
1
2
3
[log] Lysozyme dilution (0=1ug/ml)

Fig.32.

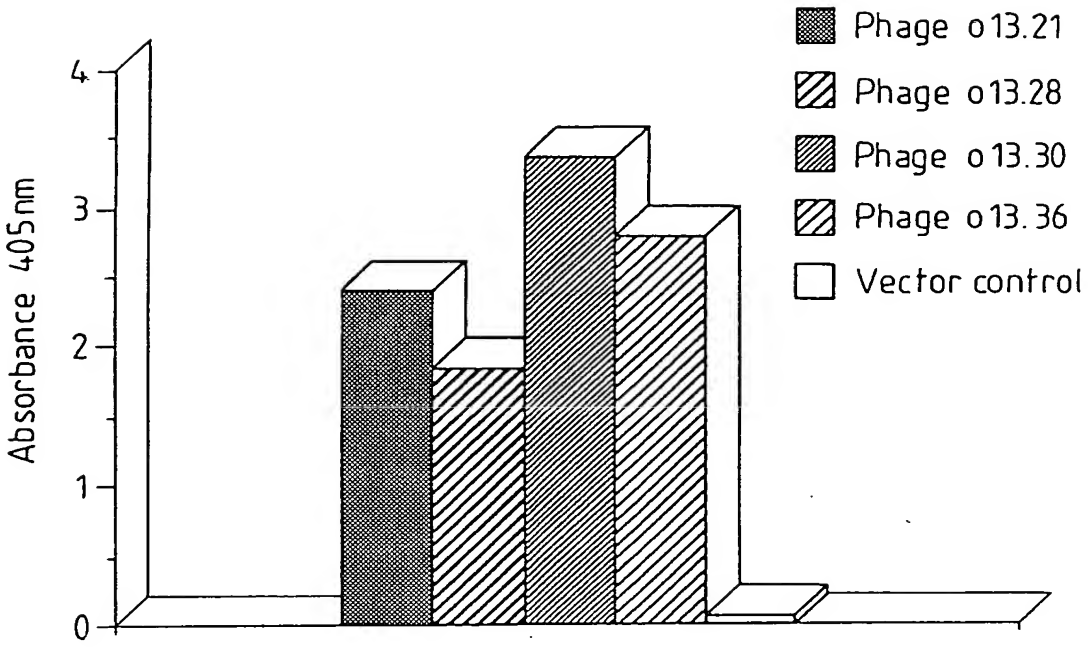

Absorbance 405nm
0
1
2
3
4
Phage o13.21
Phage o13.28
Phage o13.30
Phage o13.36
Vector control

Fig.33.

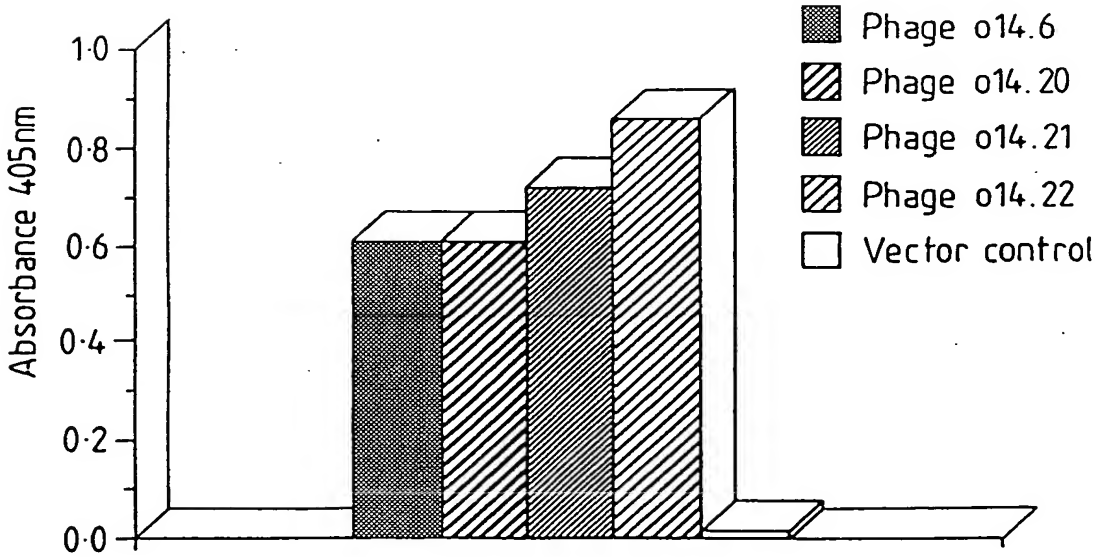

Absorbance 405nm
0·0
0·2
0·4
0·6
0·8
1·0
Phage o14.6
Phage o14.20
Phage o14.21
Phage o14.22
Vector control

Fig.34.

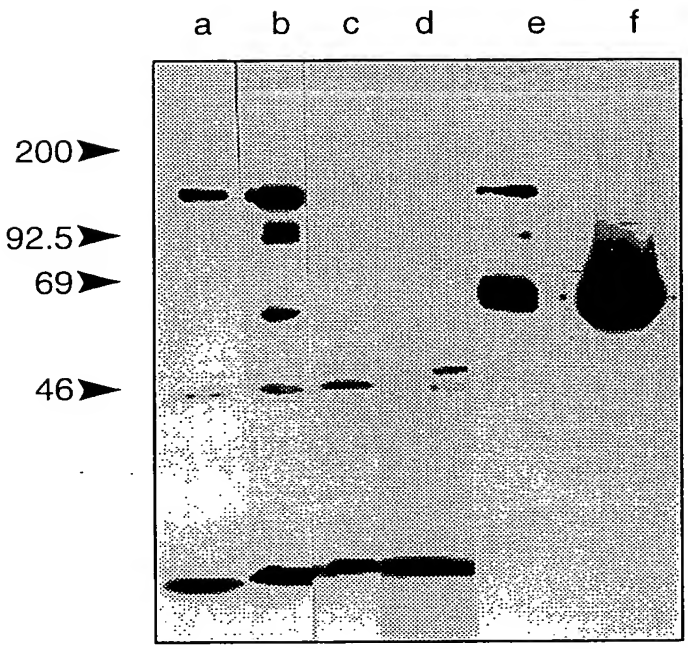


Fig.35A.

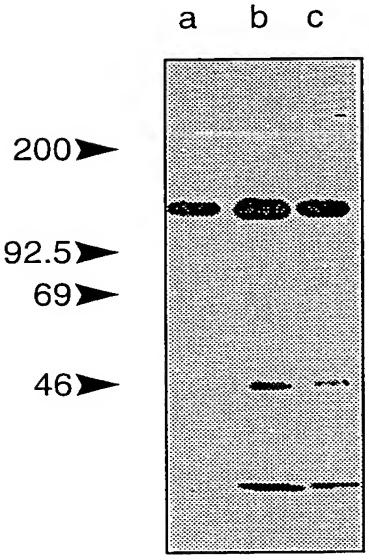


Fig.35B.

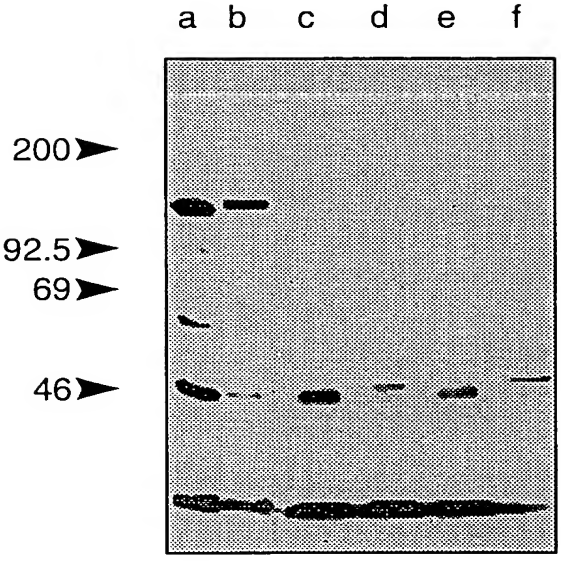


Fig.36.

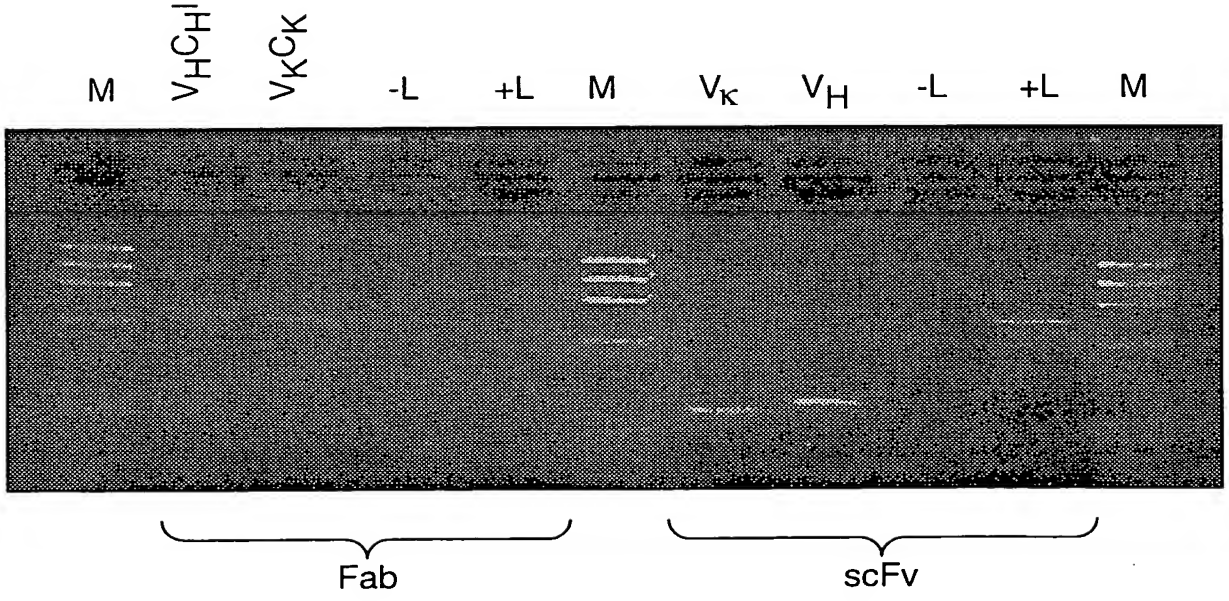
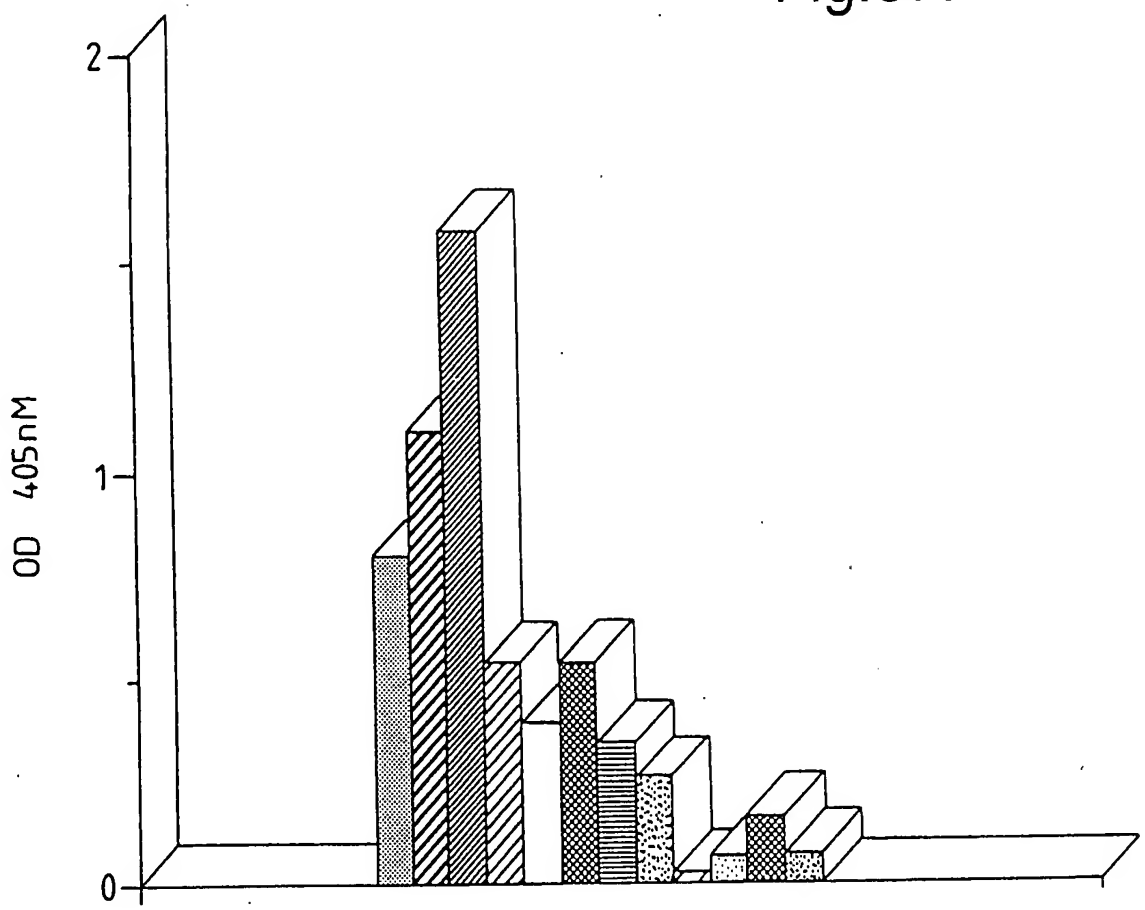


Fig.37.



fd 10x
pCAT-3 KO7 10x
pCAT-3 gIII No 3 10x
pCAT-3 gIII No 2 10x
fd 1x
pCAT-3 KO7 1x
pCAT-3 gIII No 3 1x
pCAT-3 gIII No2 1x
fd 0·1x
pCAT-3 KO7 0·1x
pCAT-3 gIII No 3 0·1x
pCAT-3 gIII No 2 0·1x

Fig.38A.

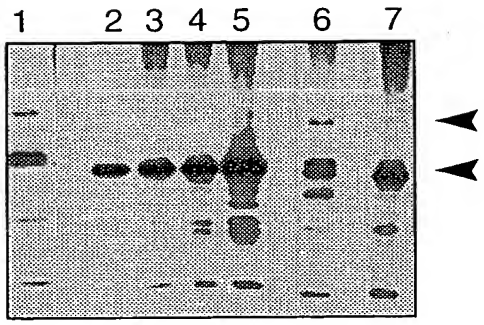


Fig.38B.

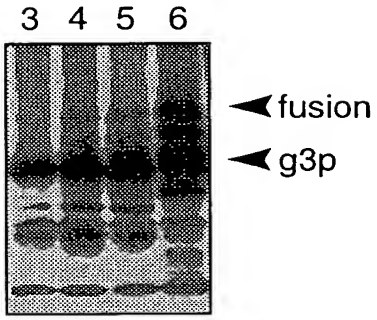


Fig.39.

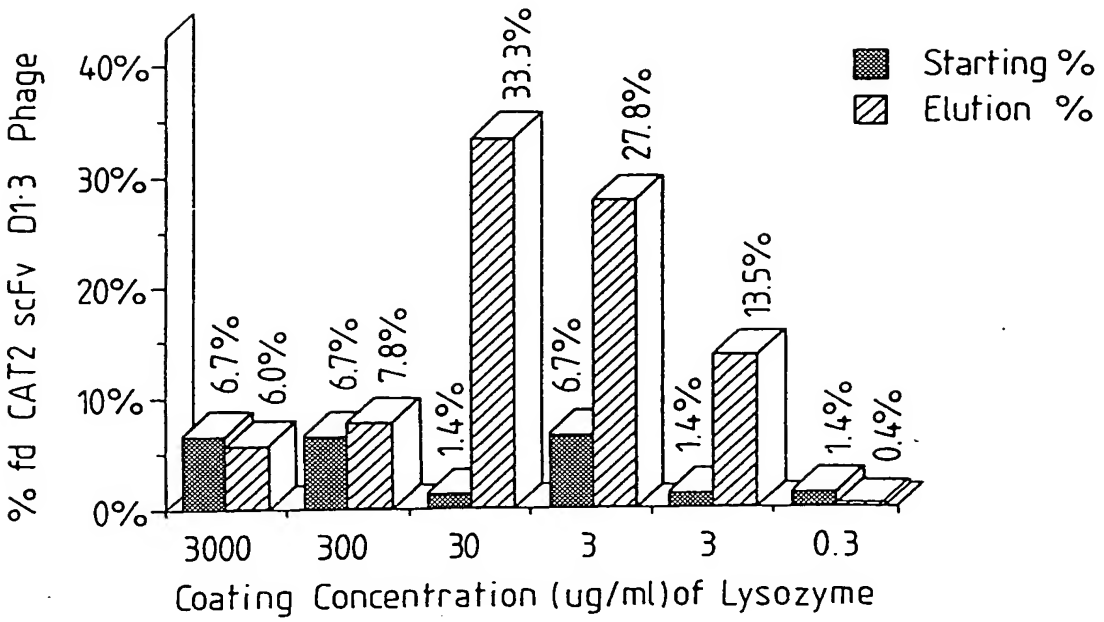

% fd CAT2 scFv D1:3 Phage
40%
30%
20%
10%
0%
6.7%
6.0%
6.7%
7.8%
1.4%
33.3%
6.7%
27.8%
1.4%
13.5%
1.4%
0.4%
3000
300
30
3
3
0.3
Coating Concentration (ug/ml)of Lysozyme
Starting %
Elution %

Fig.40.

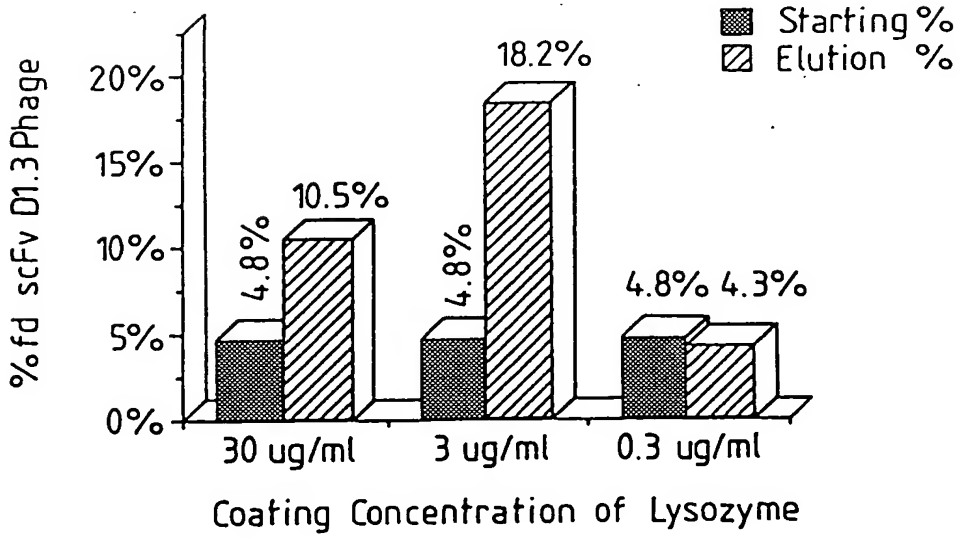

%fd scFv D1.3Phage
20%
15%
10%
5%
0%
4.8%
10.5%
4.8%
18.2%
4.8%
4.3%
30 ug/ml
3 ug/ml
0.3 ug/ml
Coating Concentration of Lysozyme
Starting %
Elution %

Fig.41.

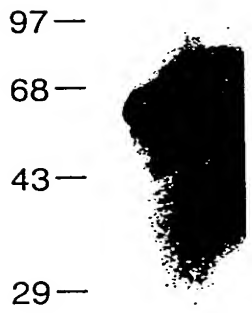


Fig.42.

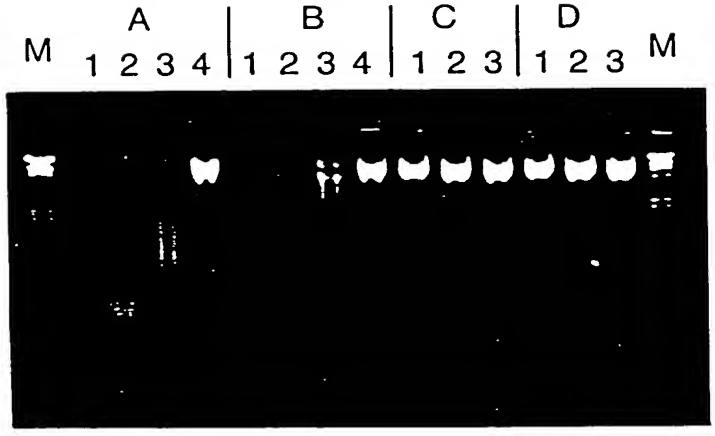


Fig.43.

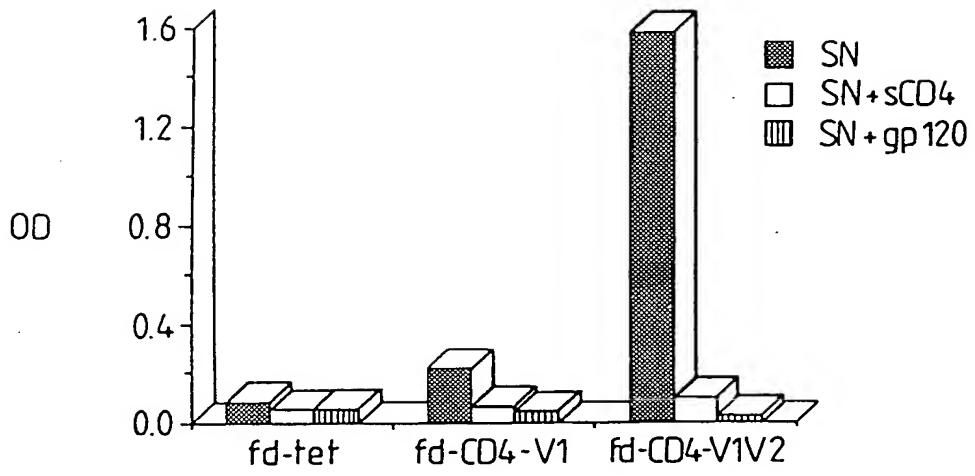
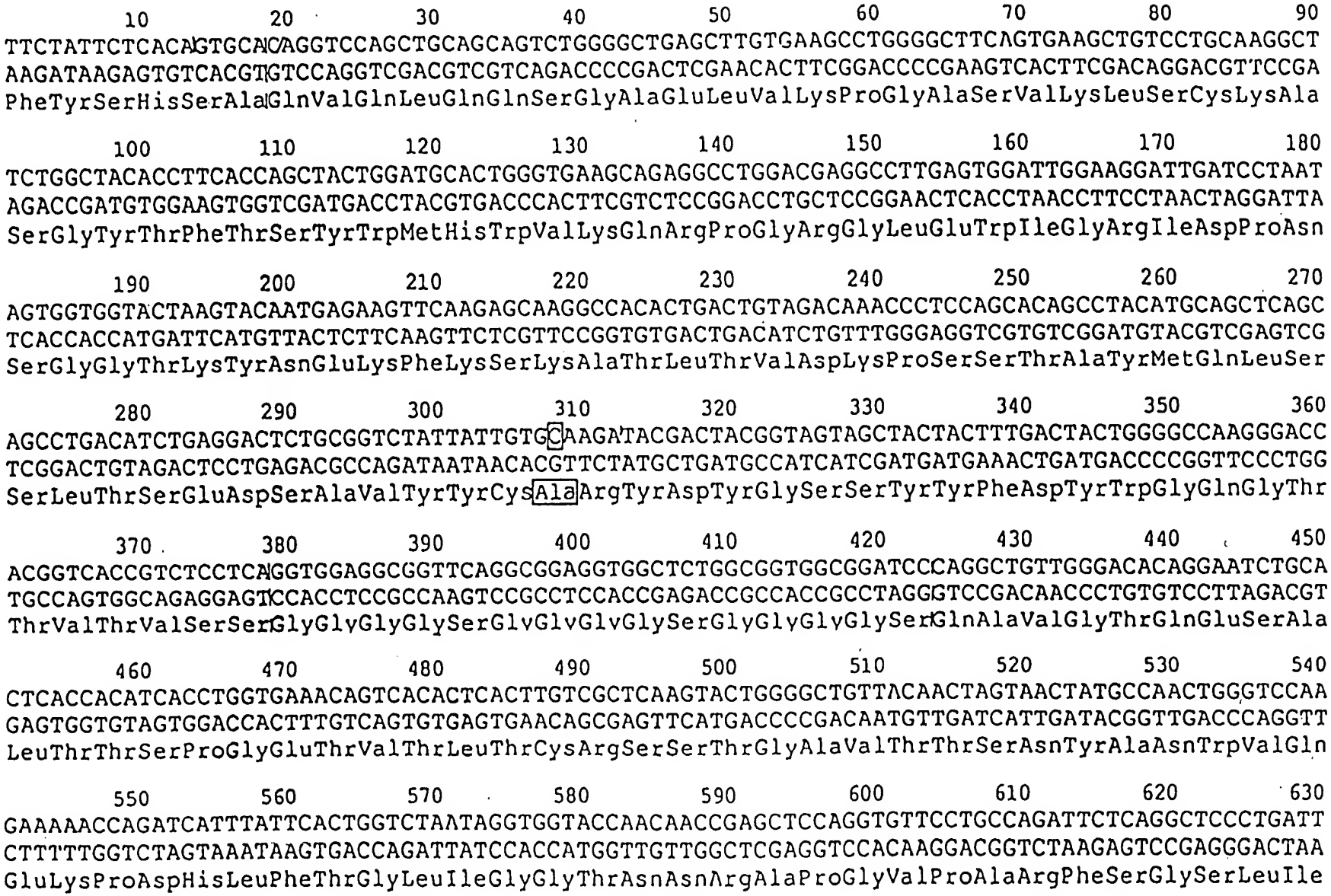


Fig.44a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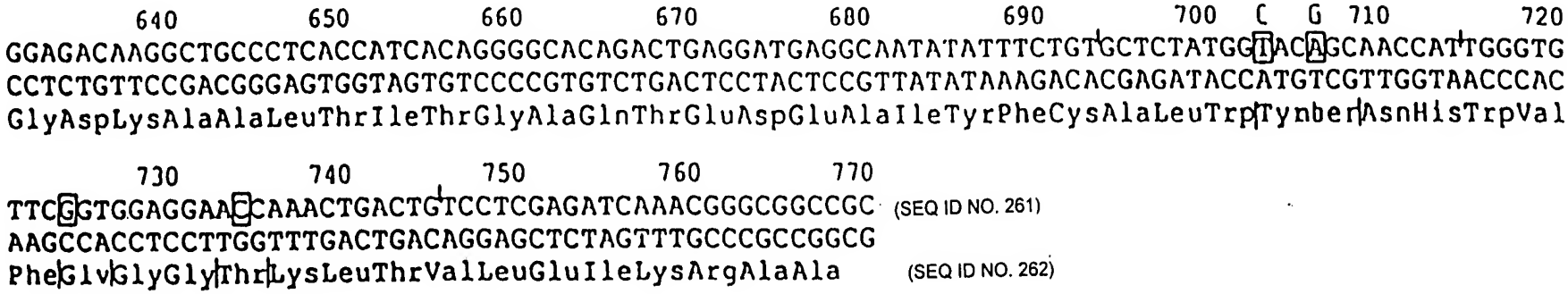
AAGATAAGAGTGTCACGTGTCCAGGTCGACGTCGTCAGACCCCGACTCGAACACTTCGGACCCCGAAGTCACTTCGACAGGACGTTCCGA
PheTyrSerHisSerAlaGlnValGlnLeuGlnGlnSerGlyAlaGluLeuValLysProGlyAlaSerValLysLeuSerCysLysAla
100 110 120 130 140 150 160 170 180
TCTGGCTACACCTTCACCAGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACGAGGCCTTGAGTGGATTGGAAGGATTGATCCTAAT
AGACCGATGTGGAAGTGGTCGATGACCTACGTGACCCACTTCGTCTCCGGACCTGCTCCGGAACTCACCTAACCTTCCTAACTAGGATTA
SerGlyTyrThrPheThrSerTyrTrpMetHisTrpValLysGlnArgProGlyArgGlyLeuGluTrpIleGlyArgIleAspProAsn
190 200 210 220 230 240 250 260 270
AGTGGTGGTACTAAGTACAATGAGAAGTTCAAGAGCAAGGCCACACTGACTGTAGACAAACCCTCCAGCACAGCCTACATGCAGCTCAGC
TCACCACCATGATTCATGTTACTCTTCAAGTTCTCGTTCCGGTGTGACTGACATCTGTTTGGGAGGTCGTGTCGGATGTACGTCGAGTCG
SerGlyGlyThrLysTyrAsnGluLysPheLysSerLysAlaThrLeuThrValAspLysProSerSerThrAlaTyrMetGlnLeuSer
280 290 300 310 320 330 340 350 360
AGCCTGACATCTGAGGACTCTGCGGTCTATTATTGTGCAAGATACGACTACGGTAGTAGCTACTACTTTGACTACTGGGGCCAAGGGACC
TCGGACTGTAGACTCCTGAGACGCCAGATAATAACACGTTCTATGCTGATGCCATCATCGATGATGAAACTGATGACCCCGGTTCCCTGG
SerLeuThrSerGluAspSerAlaValTyrTyrCysAlaArgTyrAspTyrGlySerSerTyrTyrPheAspTyrTrpGlyGlnGlyThr
370 380 390 400 410 420 430 440 450
ACGGTCACCGTCTCCTCAGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCCCAGGCTGTTGGGACACAGGAATCTGCA
TGCCAGTGGCAGAGGAGTCCACCTCCGCCAAGTCCGCCTCCACCGAGACCGCCACCGCCTAGGGTCCGACAACCCTGTGTCCTTAGACGT
ThrValThrValSerSerGlyGlyGlyGlySerGlyGlyGlyGlySerGlyGlyGlyGlySerGlnAlaValGlyThrGlnGluSerAla
460 470 480 490 500 510 520 530 540
CTCACCACATCACCTGGTGAAACAGTCACACTCACTTGTCGCTCAAGTACTGGGGCTGTTACAACTAGTAACTATGCCAACTGGGTCCAA
GAGTGGTGTAGTGGACCACTTTGTCAGTGTGAGTGAACAGCGAGTTCATGACCCCGACAATGTTGATCATTGATACGGTTGACCCAGGTT
LeuThrThrSerProGlyGluThrValThrLeuThrCysArgSerSerThrGlyAlaValThrThrSerAsnTyrAlaAsnTrpValGln
550 560 570 580 590 600 610 620 630
GAAAAACCAGATCATTTATTCACTGGTCTAATAGGTGGTACCAACAACCGAGCTCCAGGTGTTCCTGCCAGATTCTCAGGCTCCCTGATT
CTTTTTGGTCTAGTAAATAAGTGACCAGATTATCCACCATGGTTGTTGGCTCGAGGTCCACAAGGACGGTCTAAGAGTCCGAGGGACTAA
GluLysProAspHisLeuPheThrGlyLeuIleGlyGlyThrAsnAsnArgAlaProGlyValProAlaArgPheSerGlySerLeuIle

Fig.44 b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CCTCTGTTCCGACGGGAGTGGTAGTGTCCCCGTGTCTGACTCCTACTCCGTTATATAAAGACACGAGATACCATGTCGTTGGTAACCCAC
GlyAspLysAlaAlaLeuThrIleThrGlyAlaGlnThrGluAspGluAlaIleTyrPheCysAlaLeuTrpTynberAsnHisTrpVal
730 740 750 760 770
TTCGGTGGAGGAACCAAACTGACTGTCCTCGAGATCAAACGGGCGGCCGC (SEQ ID NO. 261)
AAGCCACCTCCTTGGTTTGACTGACAGGAGCTCTAGTTTGCCCGCCGGCG
PheGlvGlyGlyThrLysLeuThrValLeuGluIleLysArgAlaAla (SEQ ID NO. 262)

Fig.45.

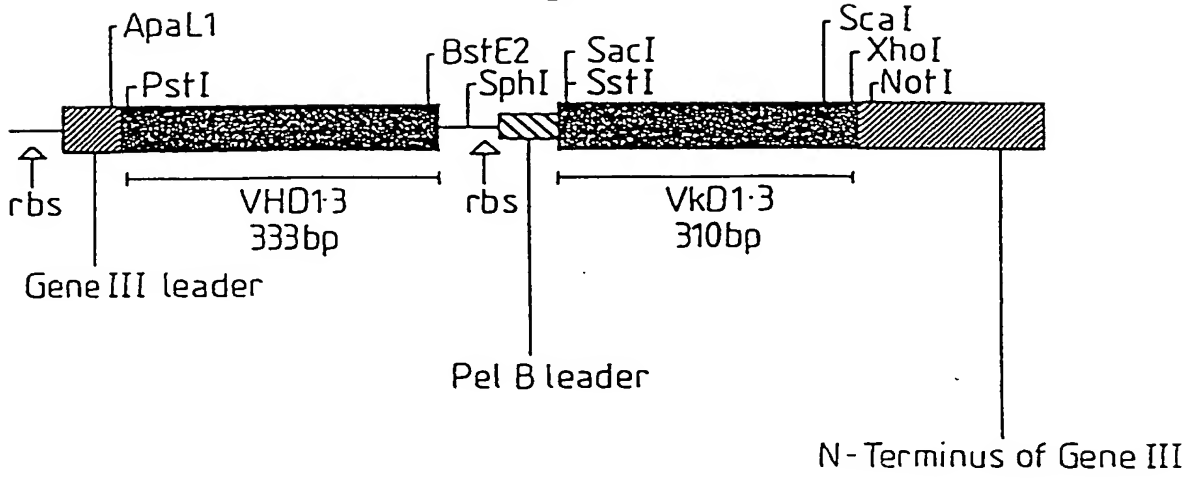

ApaL1
PstI
BstE2
SphI
SacI
SstI
ScaI
XhoI
NotI
rbs
VHD1·3
333bp
rbs
VkD1·3
310bp
Gene III leader
Pel B leader
N-Terminus of Gene III

Fig.46.

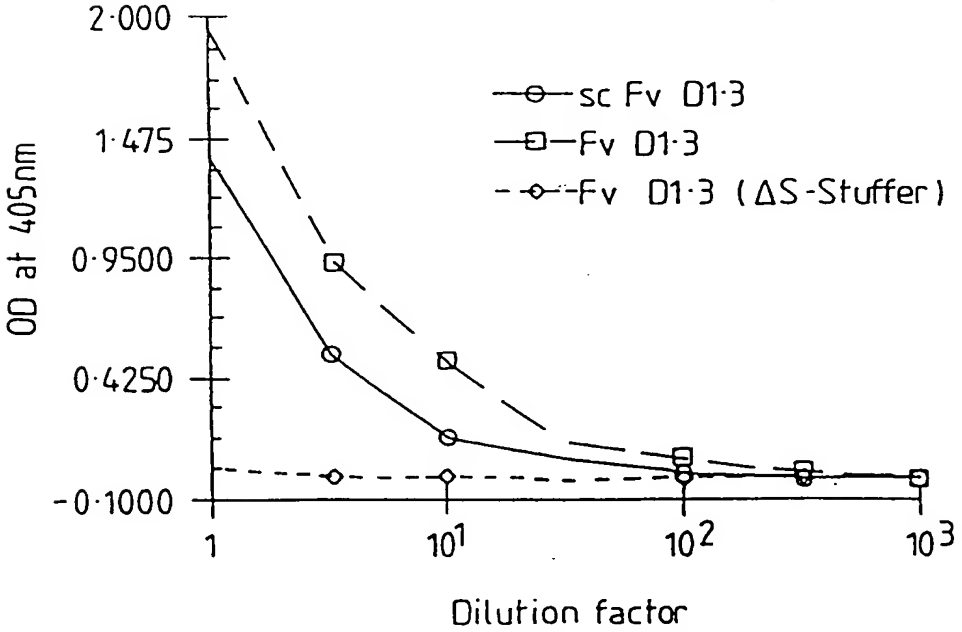

2·000
1·475
0·9500
0·4250
-0·1000
OD at 405nm
1
10¹
10²
10³
Dilution factor
sc Fv D1·3
Fv D1·3
Fv D1·3 (ΔS-Stuffer)

Fig.47.

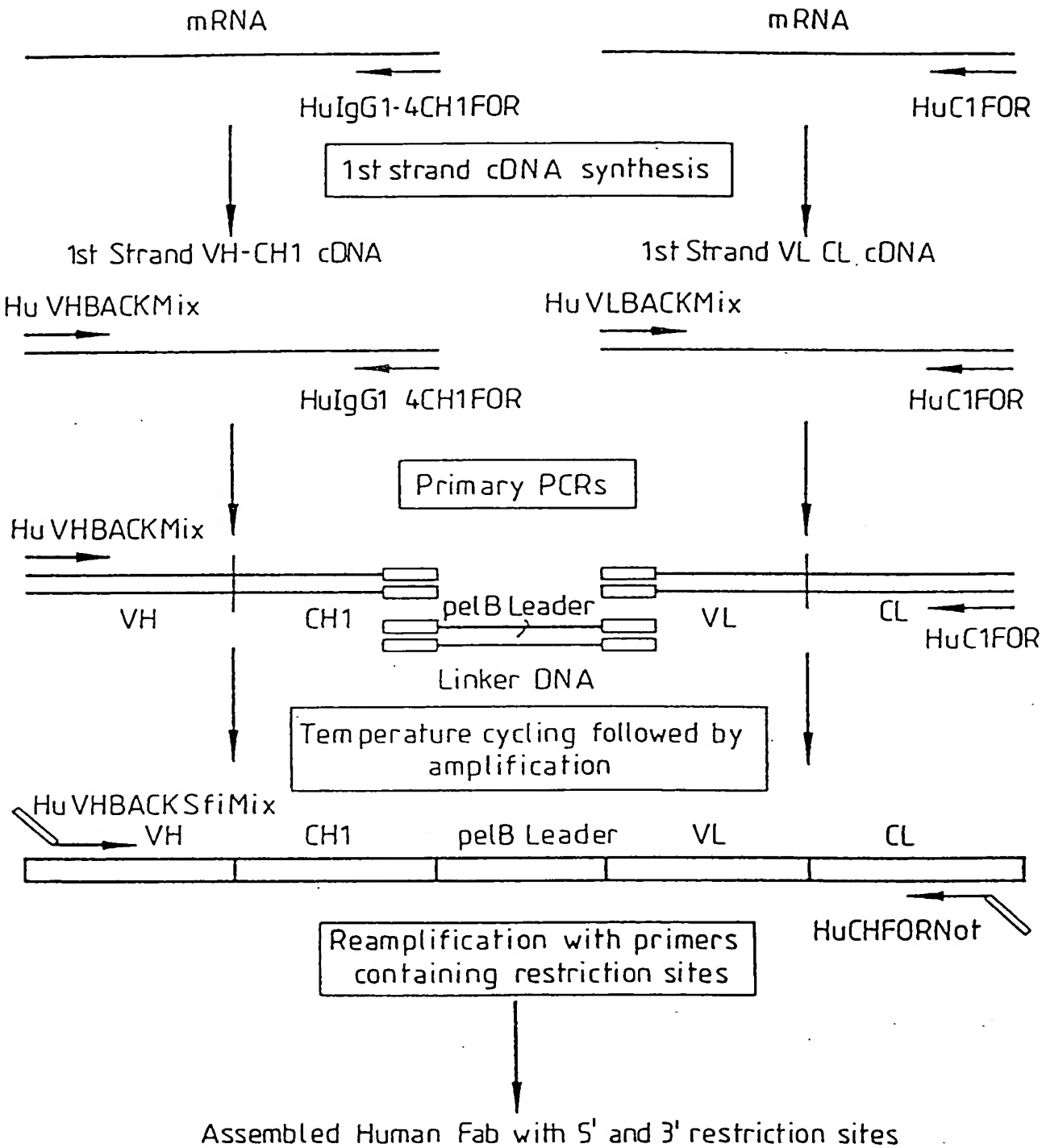

mRNA
mRNA
HuIgG1-4CH1FOR
HuC1FOR
1st strand cDNA synthesis
1st Strand VH-CH1 cDNA
1st Strand VL CL cDNA
Hu VHBACKMix
Hu VLBACKMix
HuIgG1 4CH1FOR
HuC1FOR
Primary PCRs
Hu VHBACKMix
VH
CH1
pelB Leader
VL
CL
HuC1FOR
Linker DNA
Temperature cycling followed by amplification
Hu VHBACKSfiMix
VH
CH1
pelB Leader
VL
CL
HuCHFORNot
Reamplification with primers containing restriction sites
Assembled Human Fab with 5' and 3' restriction sites

Fig. 48a

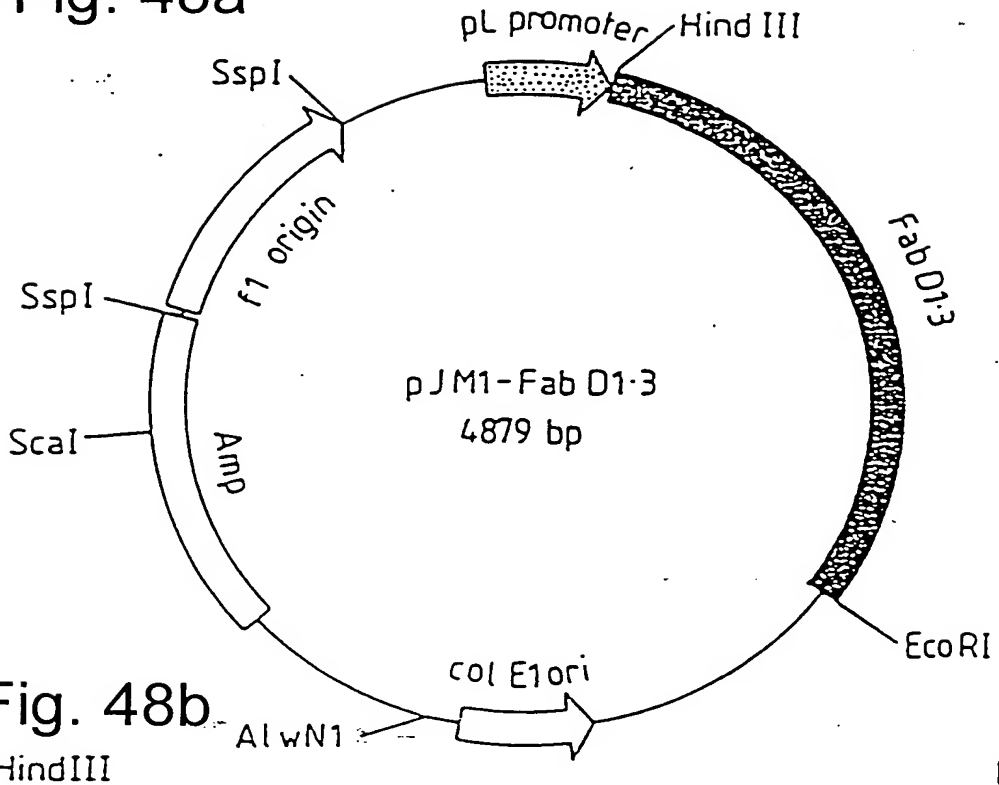


Fig. 48b

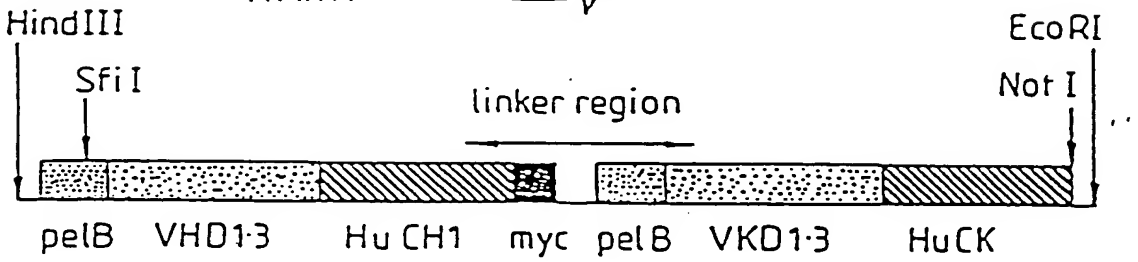


Fig. 48c

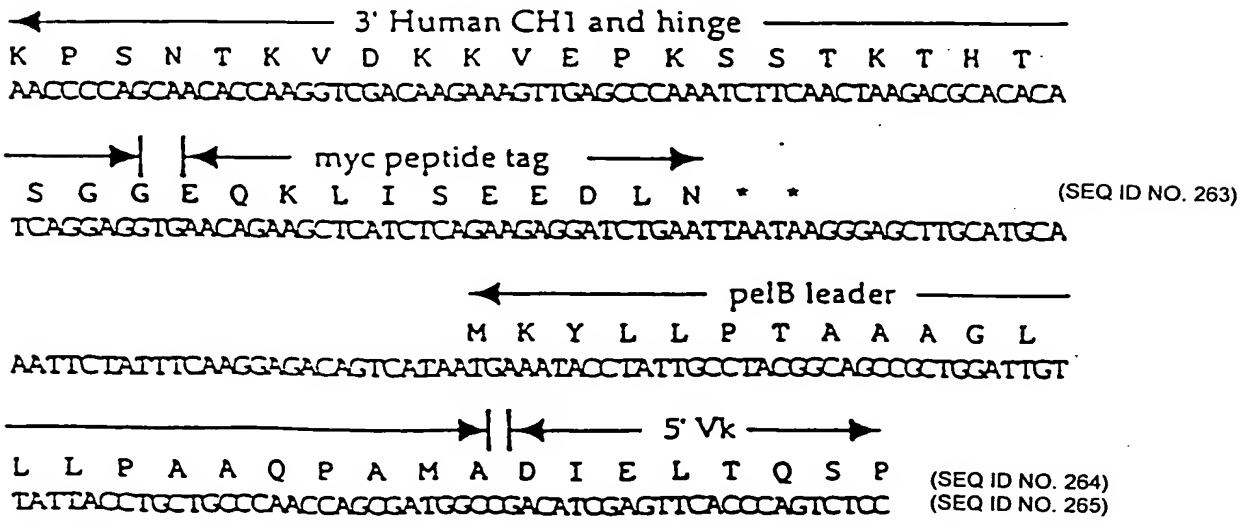


Fig.49.

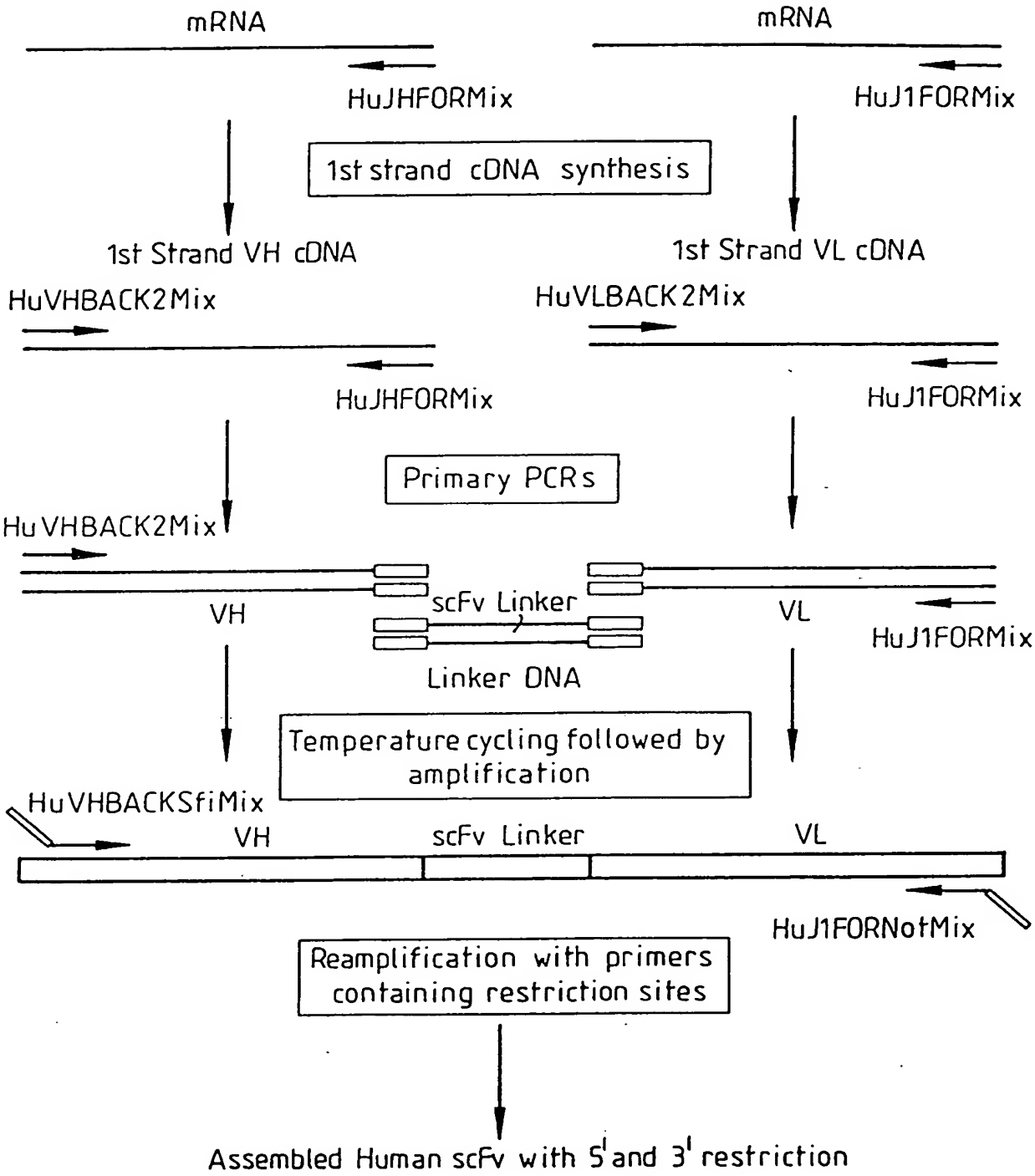

mRNA
mRNA
HuJHFORMix
HuJ1FORMix
1st strand cDNA synthesis
1st Strand VH cDNA
1st Strand VL cDNA
HuVHBACK2Mix
HuVLBACK2Mix
HuJHFORMix
HuJ1FORMix
Primary PCRs
HuVHBACK2Mix
VH
scFv Linker
VL
HuJ1FORMix
Linker DNA
Temperature cycling followed by amplification
HuVHBACKSfiMix
VH
scFv Linker
VL
HuJ1FORNotMix
Reamplification with primers containing restriction sites
Assembled Human scFv with 5' and 3' restriction

Fig.50a

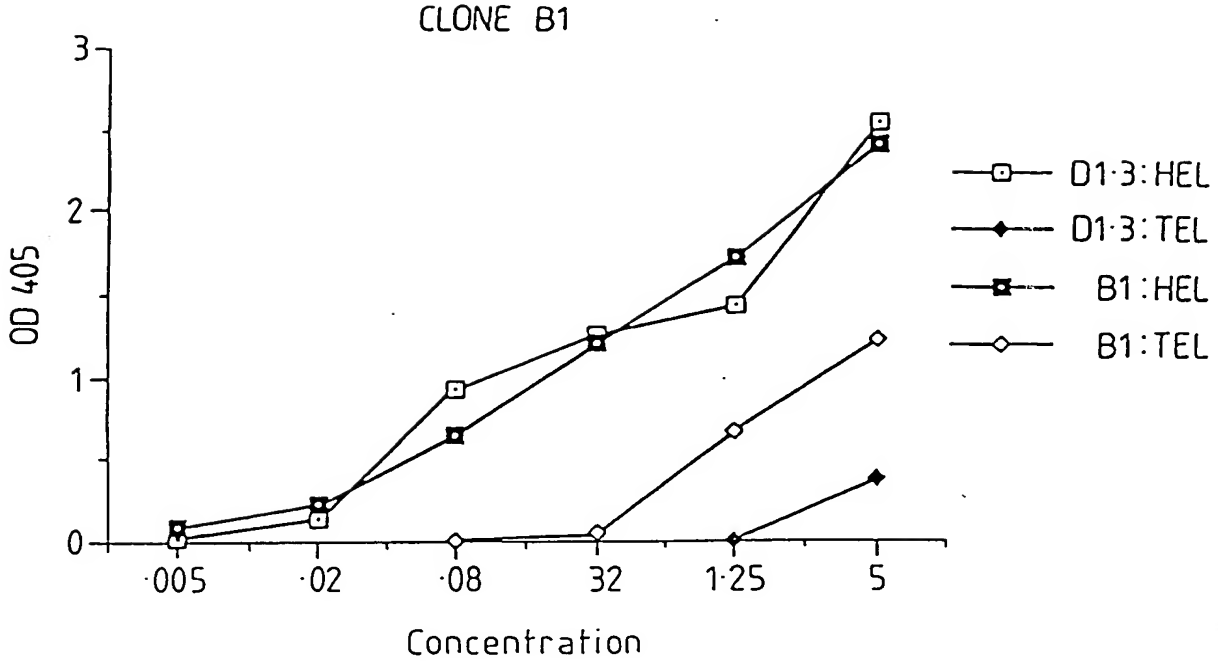


Fig.50b

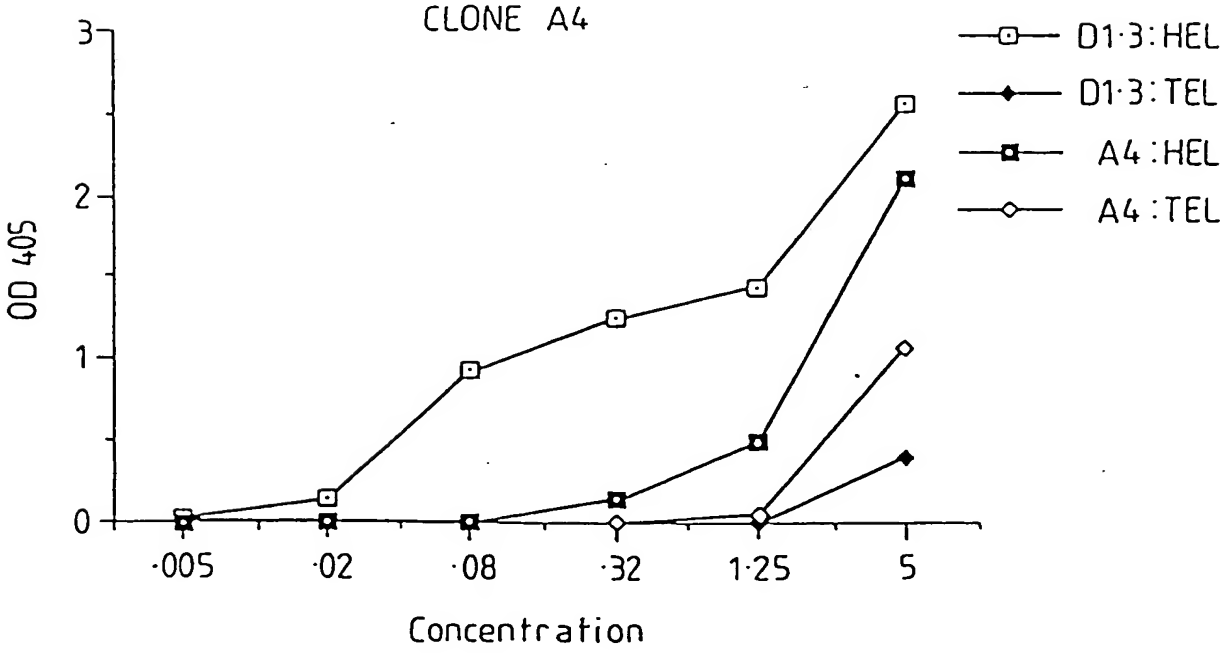


Fig.51.

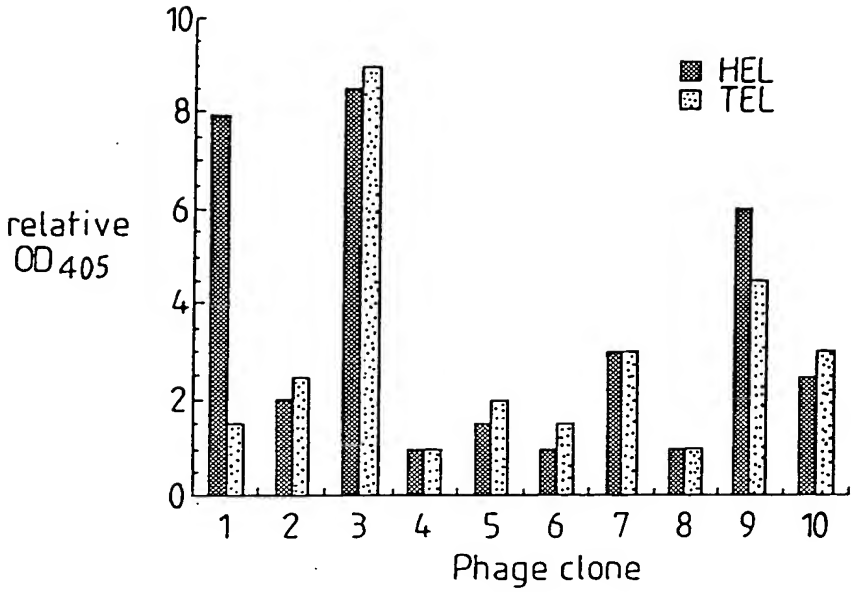

relative
OD405
10
8
6
4
2
0
HEL
TEL
1
2
3
4
5
6
7
8
9
10
Phage clone

Fig.53.

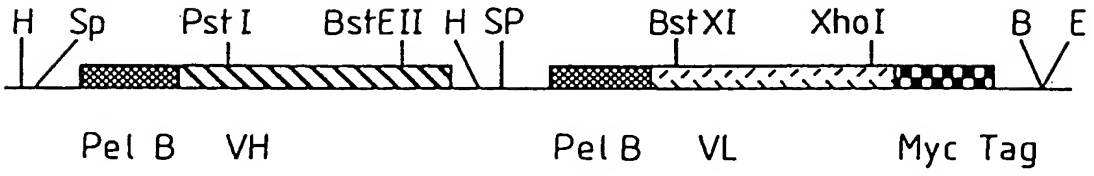
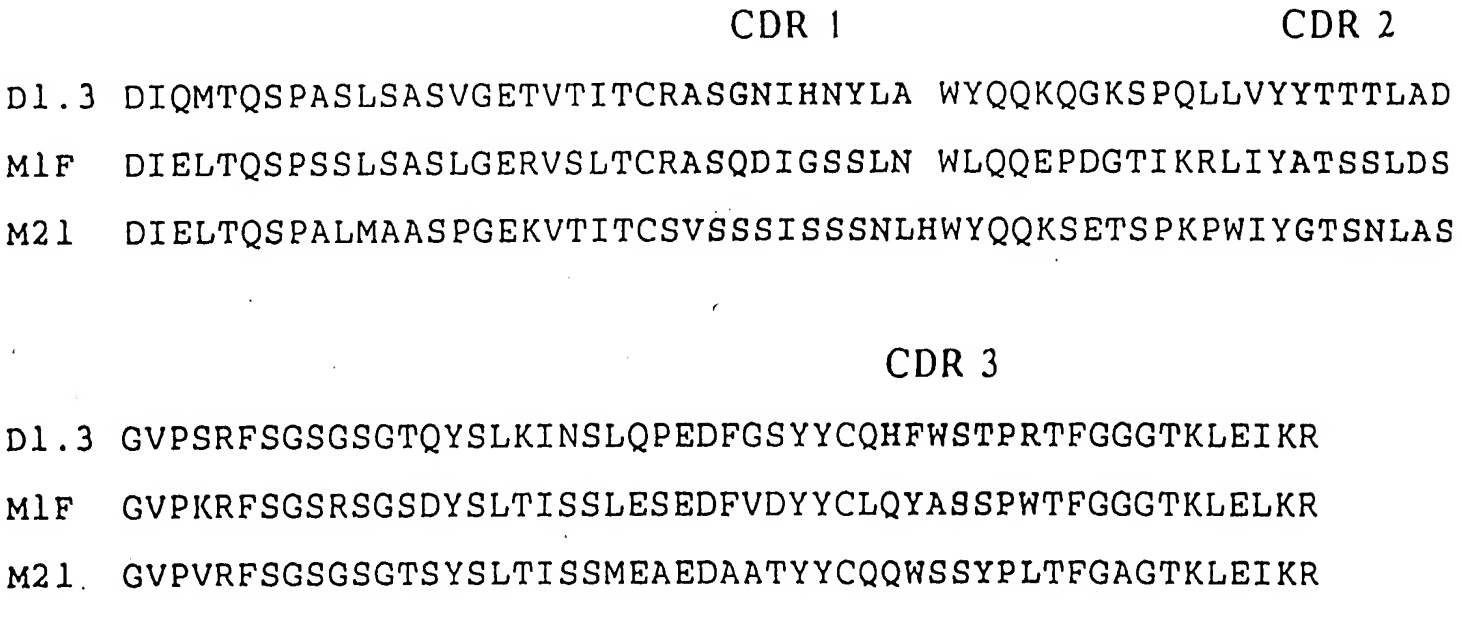

H
Sp
PstI
BstEII
H
SP
BstXI
XhoI
B
E
Pel B
VH
Pel B
VL
Myc Tag

Fig.52.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CDR 2
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M1F DIELTQSPSSLSASLGERVSLTCRASQDIGSSLN WLQQEPDGTIKRLIYATSSLDS
M21 DIELTQSPALMAASPGEKVTITCSVSSSISSSNLHWYQQKSETSPKPWIYGTSNLAS
CDR 3
D1.3 GVPSRFSGSGSGTQYSLKINSLQPEDFGSYYCQHFWSTPRTFGGGTKLEIKR
M1F GVPKRFSGSRSGSDYSLTISSLESEDFVDYYCLQYASSPWTFGGGTKLELKR
M21 GVPVRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSYPLTFGAGTKLEIKR